

Figure 1A. Peptide-linker- β chain construct

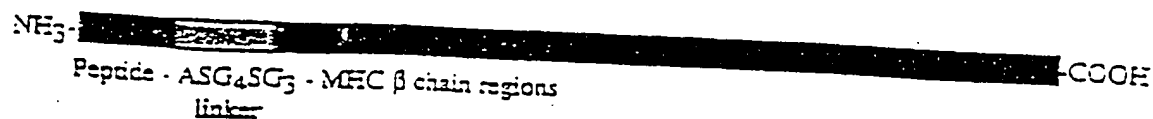


Figure 1B. Schematic view of peptide-linked MHC binding groove

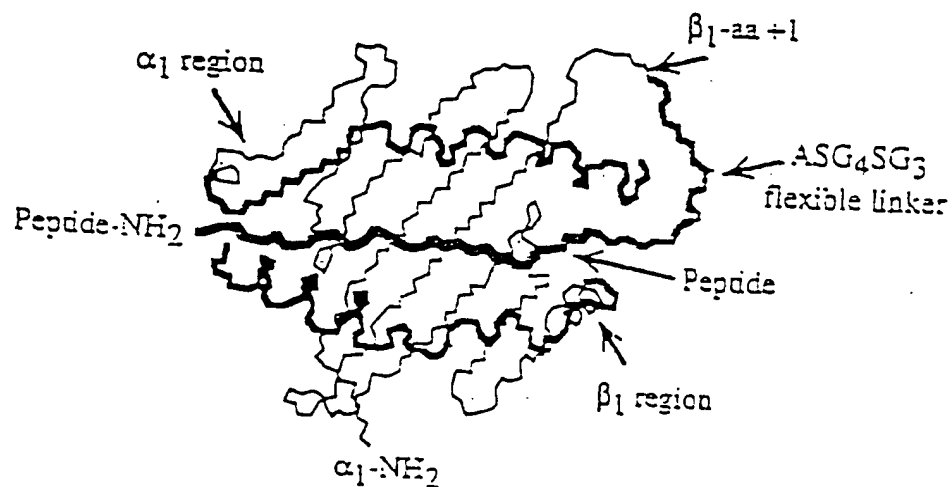
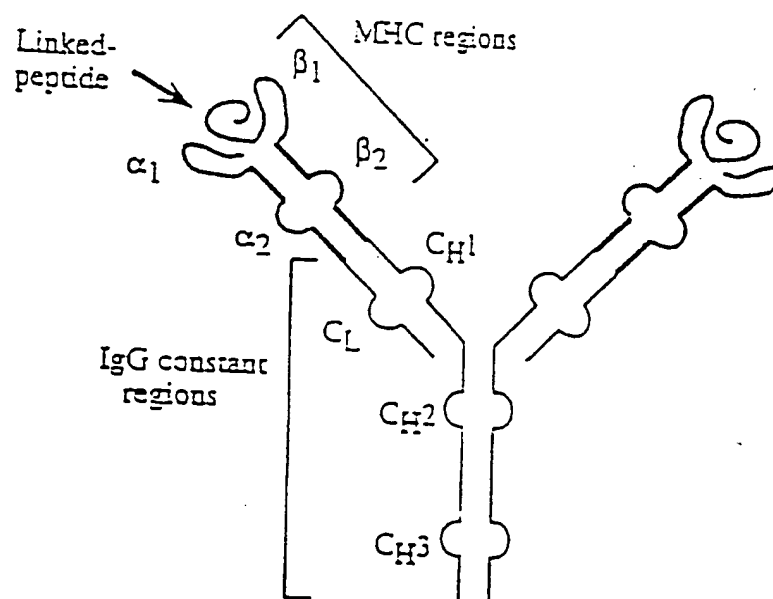
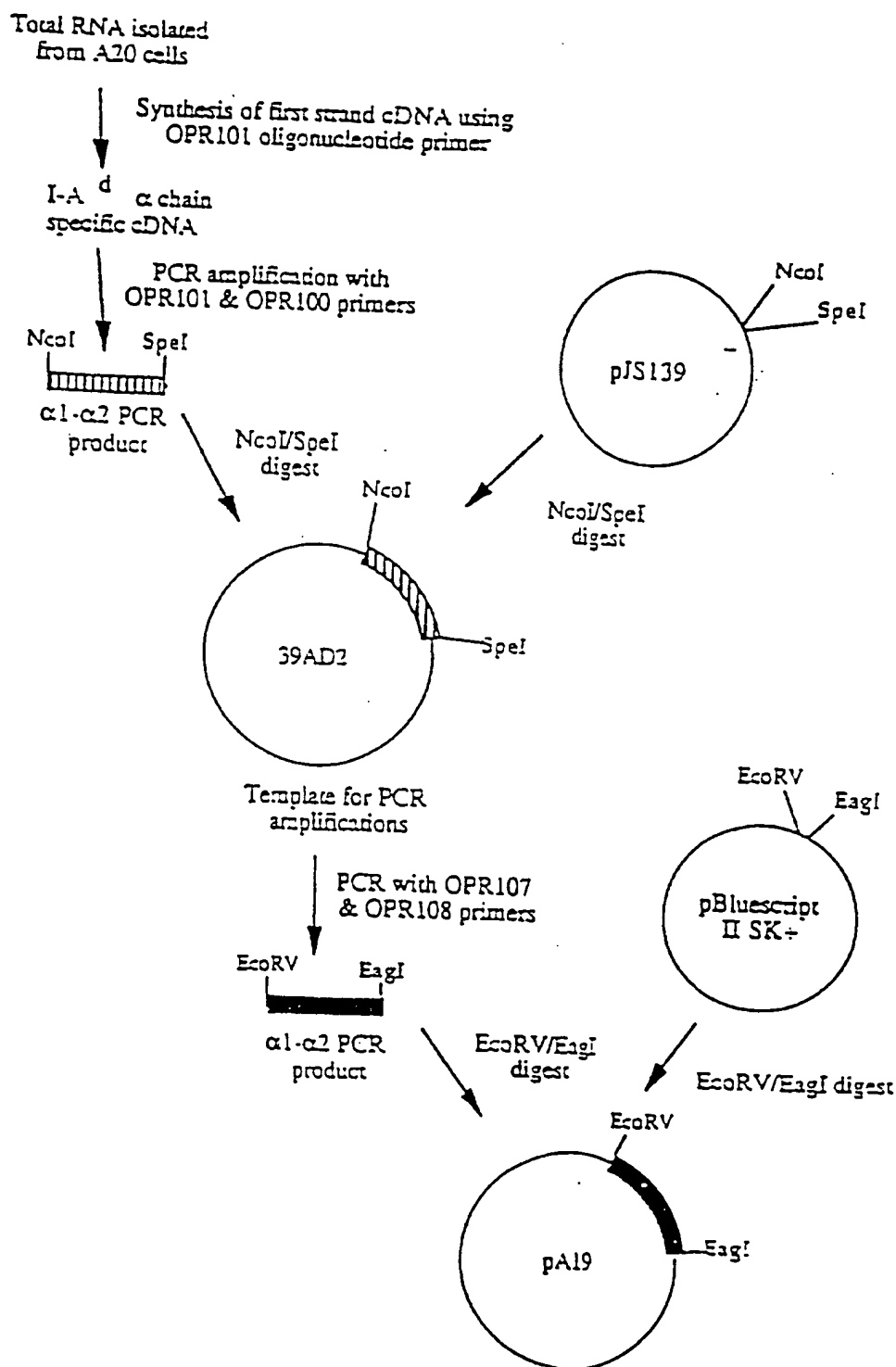


Figure 1C. Schematic view of soluble peptide-linked MHC-IgG C-region fusion protein

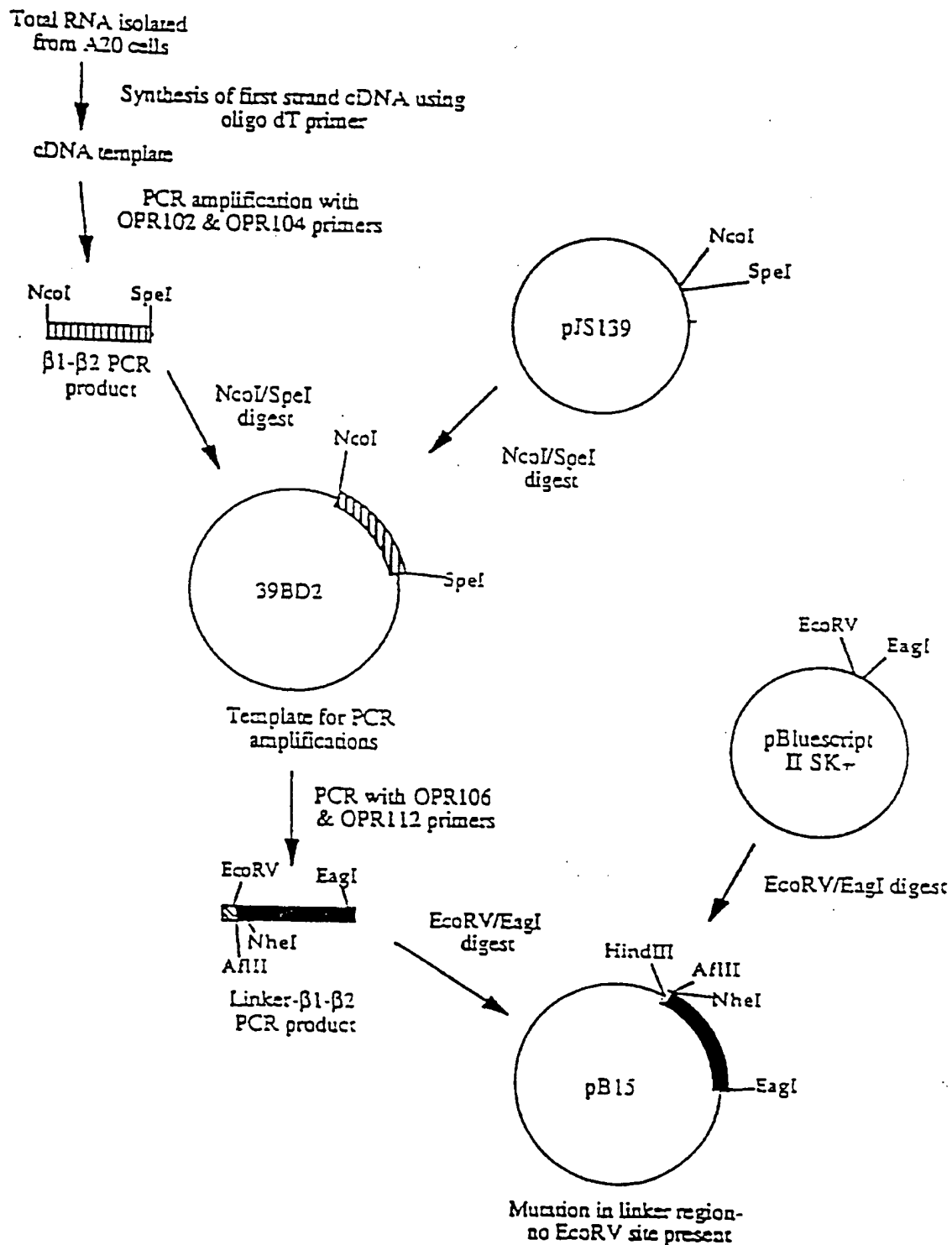


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Figure 2: I-A^d α chain cloning scheme

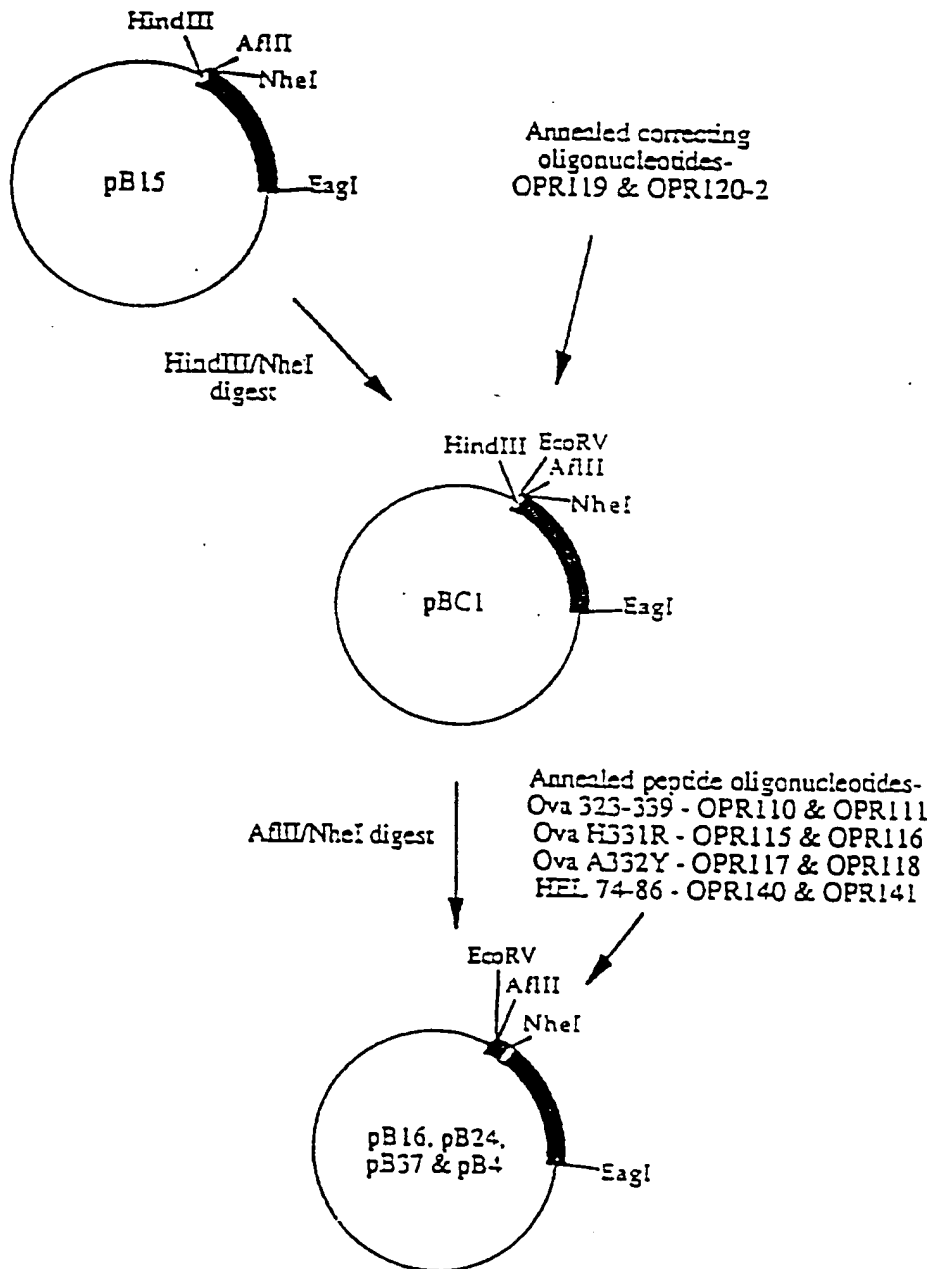
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Figure 3: I-A^d β chain cloning scheme



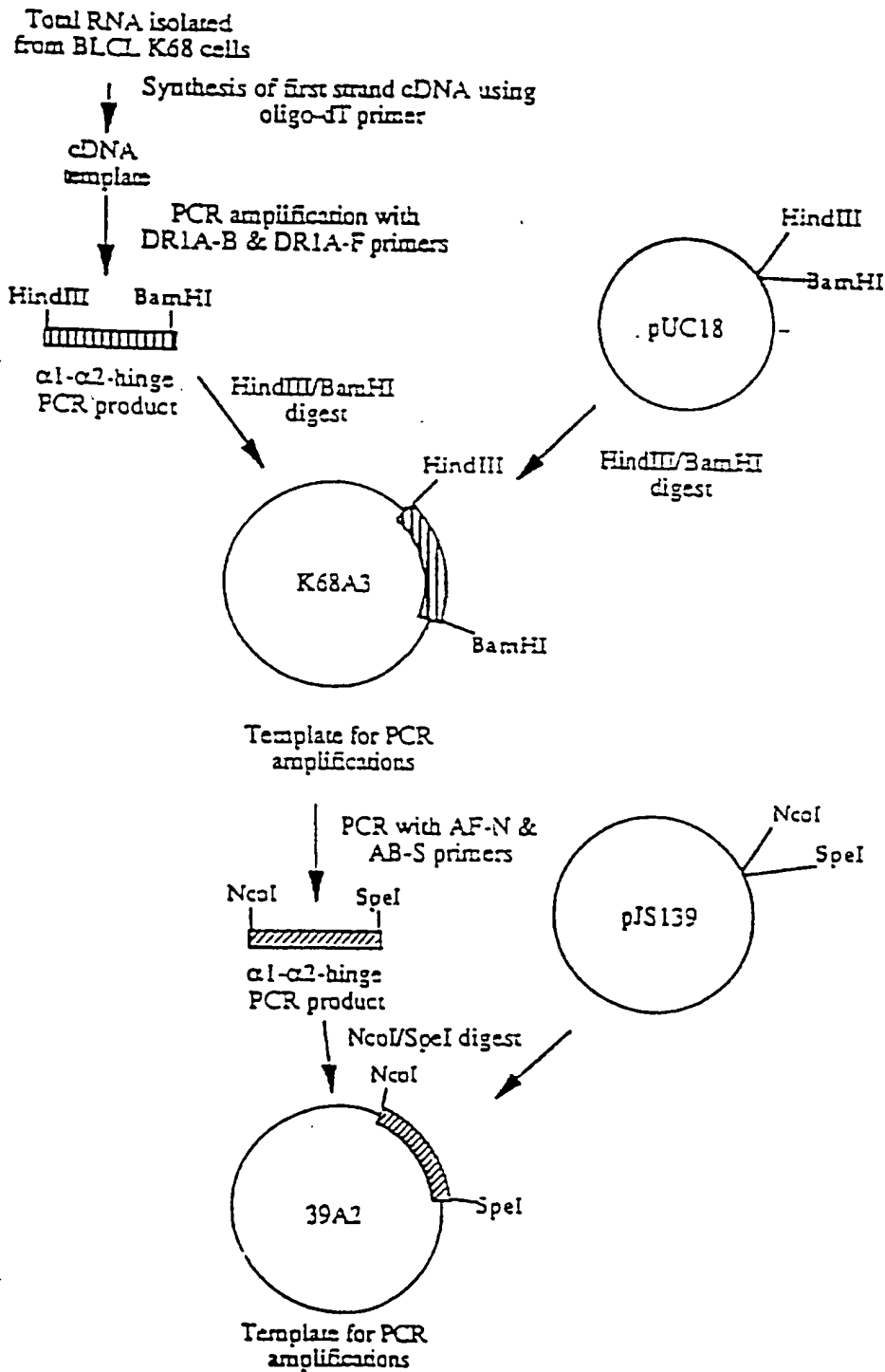
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Figure 3: cont.



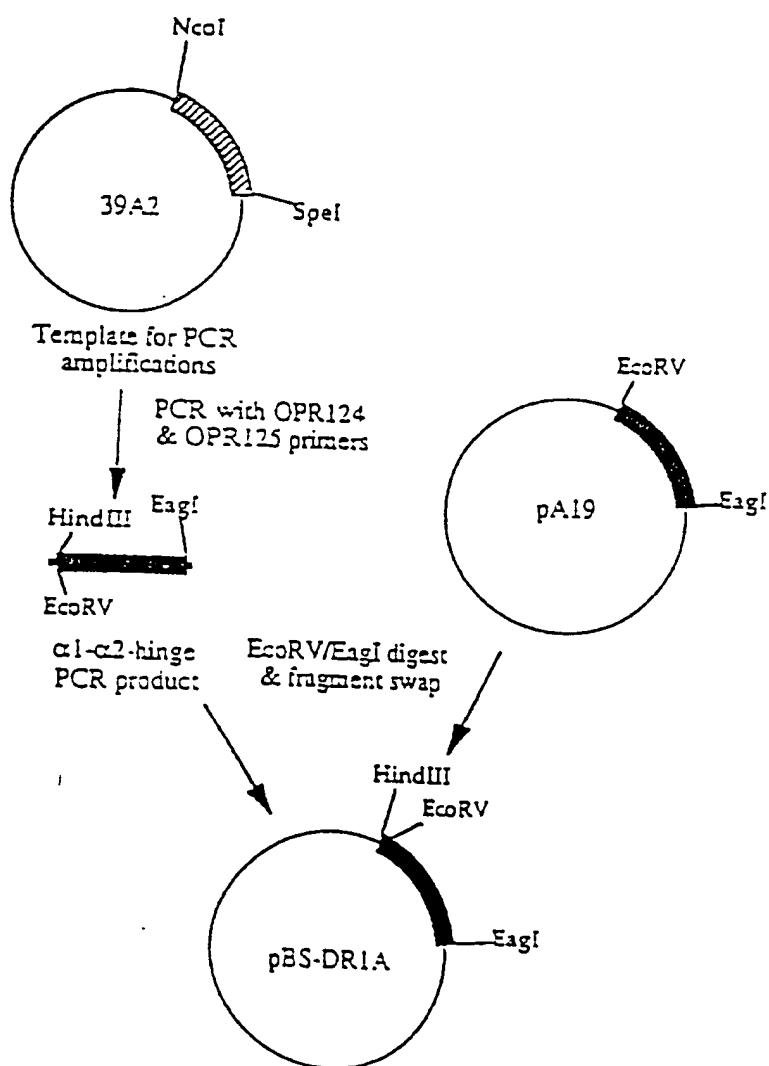
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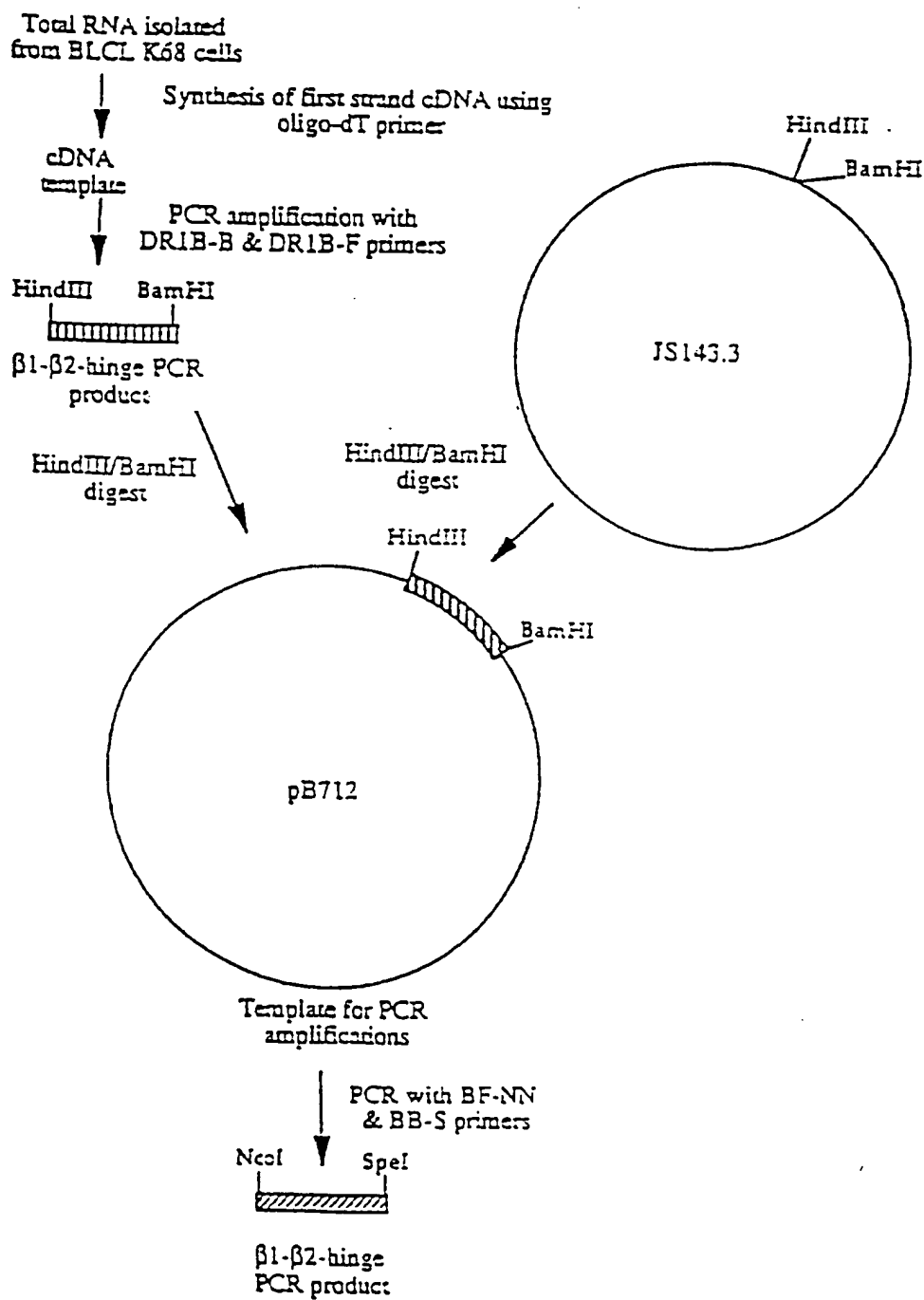
Figure 4: HLA-DR1 α chain cloning scheme

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Figure 4: cont.

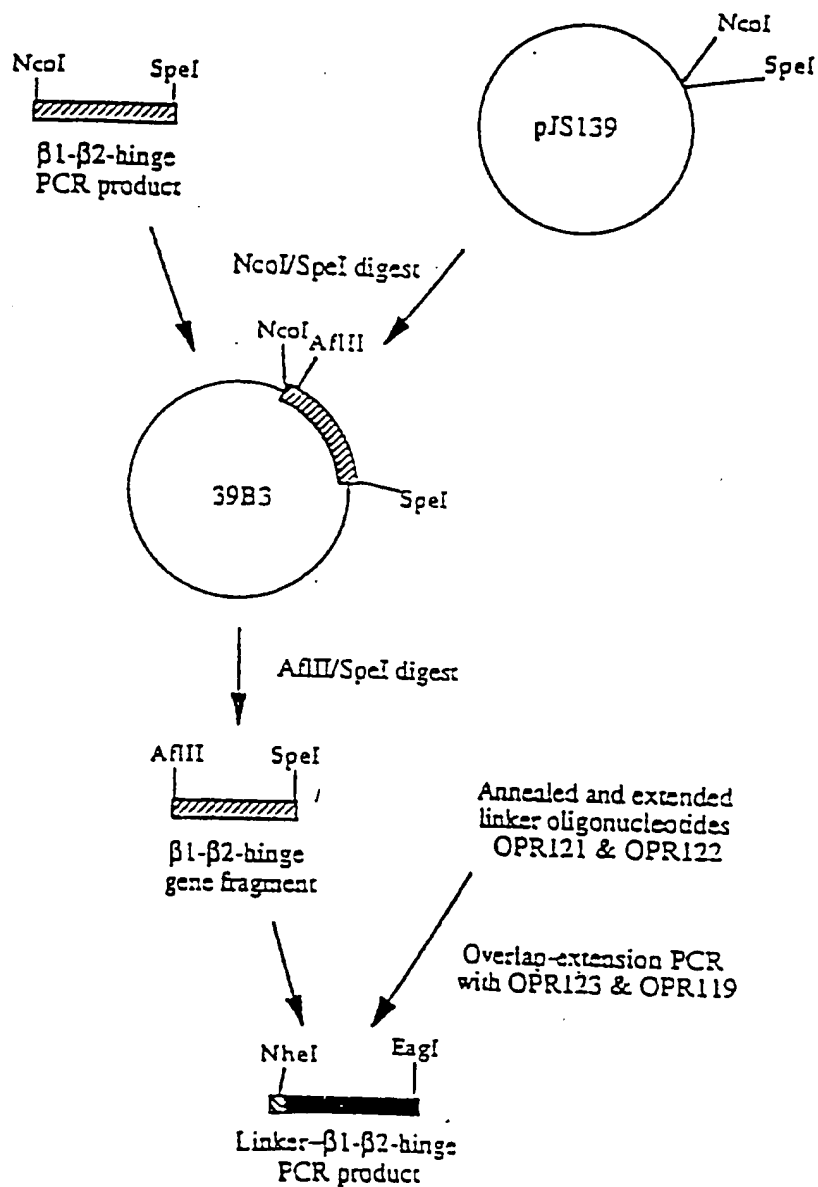


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Figure 5: HLA-DR1 β chain cloning scheme

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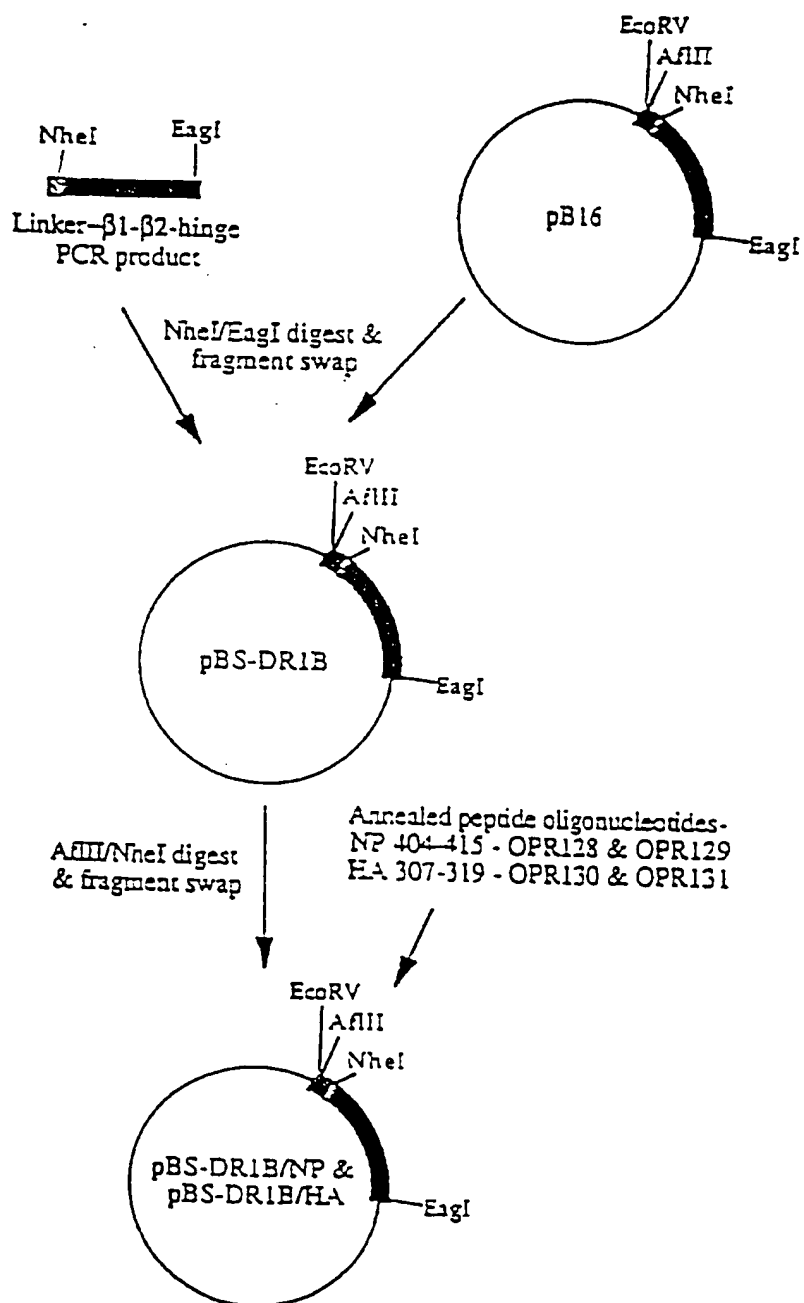
Figure 5: cont.



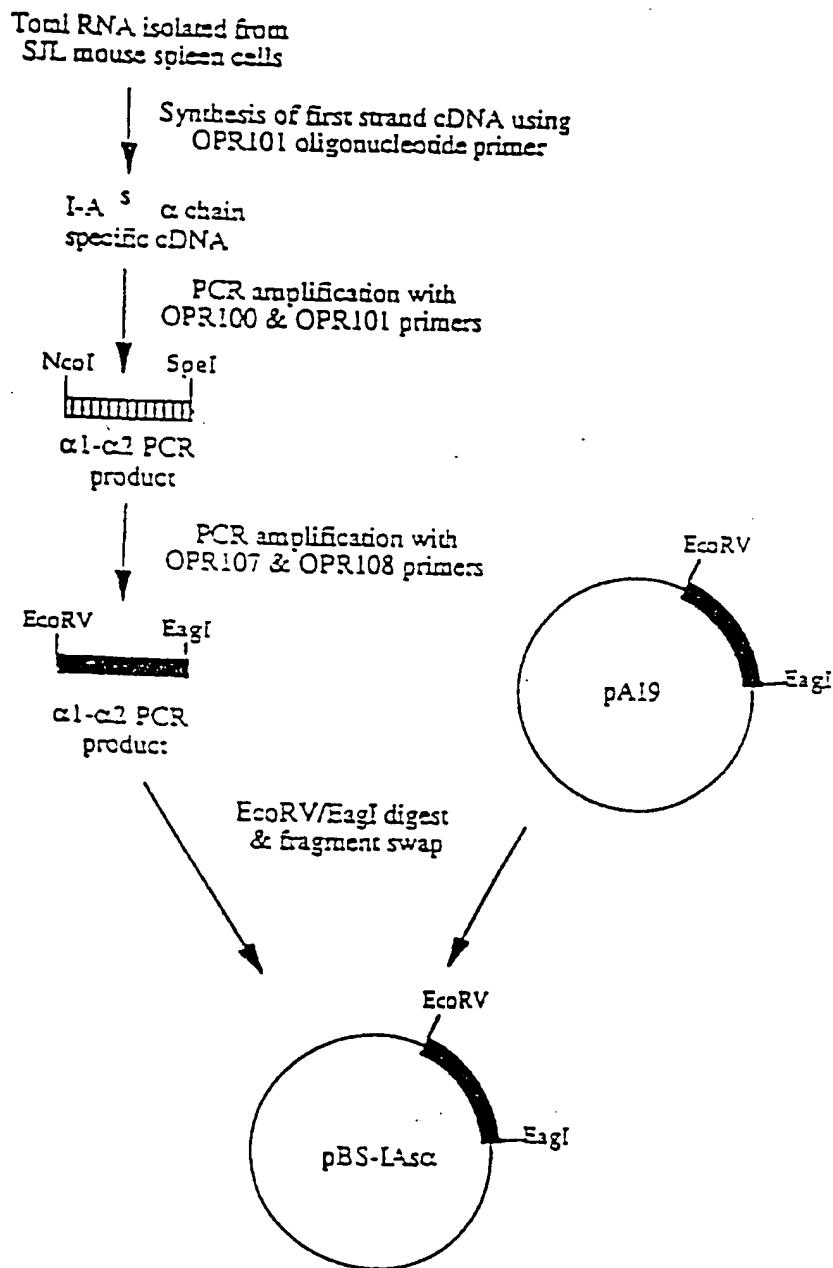
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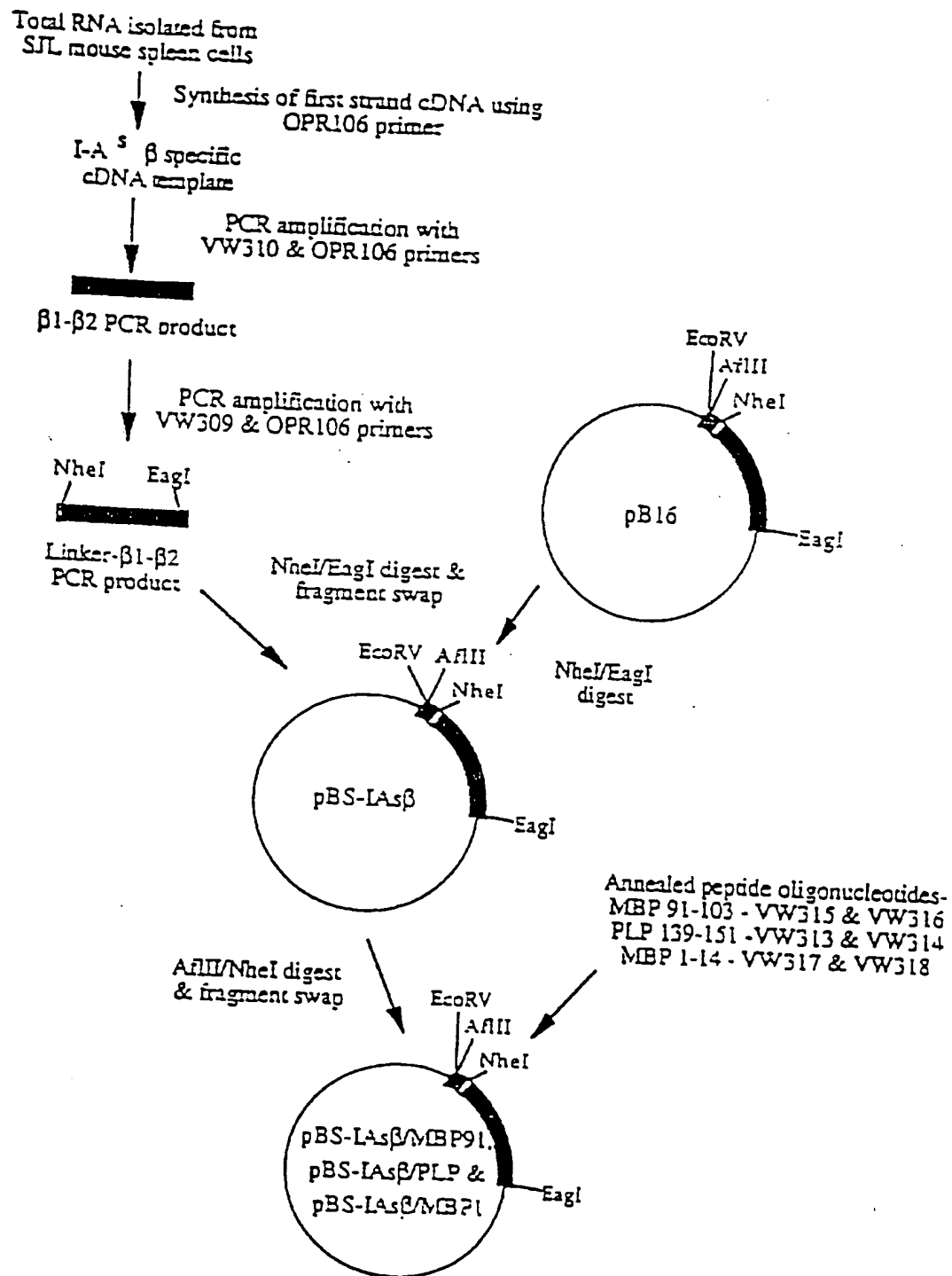
Figure 5: cont.



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Figure 6: I-A^S α chain cloning scheme

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Figure 7: I-A^S β chain cloning scheme

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Figure 8 - Oligonucleotides used in constructing MHC vectors

I-A^d/I-A^s PCR primers and cloning oligonucleotides (restriction site are underlined).

OPR100

5'-GGG GGG GCC ATG GCC GAA GAC GAC ATT GAG GCC GAC-3'

OPR101

5'-GGG GCG ACT AGT CCA GTG TTT CAG AAC CGG CTC-3'

OPR107

5'-CCC CCC GAT ATC TCA GGT TCC AGC AGT GGA GAC GAC ATT GAG
GCC G-3'

OPR108

5'-CCC CCC CGG CCG CTA CTT ACG TTT CCA GTG TTT CAG AAC CGG
C-3'

OPR102

5'-GGG GGG GCC ATG GCC GGA AAC TCC GAA AGG CAT TTC G-3'

OPR104

5'-GGG GCG ACT AGT CCA CTC CAC AGT GAT GGG GC-3'

OPR106

5'-CCC CCC CGG CCG TAC CTG AGG ACC ACT CCA CAG TGA TGG-3'

OPR112

5'-CCC CCC GAT ATC ACA GGT GTC TTA AGT GCT AGC GGA GGG GGC
GGA AGC GGC GGA GGG GGA AAC TCC GAA AGG CAT TTC-3'

OPR119

5'-AGC TTG ATA TCA CAG GTG TCT TAA GTG GAG-3'

OPR120-2

5'-CTA GCT CCA CTT AAG ACA CCT GTG ATA TCA-3'

VW310

5'-TCC GGA GGC GGC GGA GAC TCC GAA AGG CAT TTC G-3'

VW309

5'-CGA TCG CTA GCG GCG GTG GTG GTT CCG GTG GCG GCG GAG-3'

OPR136

5'-CCC CCC AGG CTT CCC GGG CCA CCA TGC CCT GCA GCA GAG CTC
TG-3'

OPR139

5'-CCC CCC GAG CTC GAA TTC TCA TAA AGG CCC TGG GTG TCT G-3'

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Figure 8 - cont.

OPR132

5'-CCC CCC AAG CTT CCC GGG CCA CCA TGG CTC TGC AGA TCC CCA
GC-3'

OPR133

5'-CCC CCC ACT TAA GGT CCT TGG GCT GCT CAG CAC C-3'

OPR134

5'-CCC CCC CCA TCA CTG TGG AGT GGA GGG-3'

OPR135

5'-CCC CCC GAG CTC GAA TTC TCA CTG CAG GAG CCC TGC TGG-3'

HLA-DR1 PCR primers and cloning oligonucleotides.

DR1A-F

5'-GGG GGG AAG CTT ATG ATC AAA GAA GAA CAT GTG ATC ATC-3'

DR1A-B

5'-GCG GCG GGA TCC GTT CTC TGT AGT CTC TGG GAG AGG-3'

DR1B-F

5'-GGG GGG AAG CTT ATG GGG GAC ACC CCA CCA CGT TTC TTG TGG
CAG C-3'

AF-N

5'-GGG GGG GCC ATG GCC ATC AAA GAA GAA CAT GTG ATC ATC-3'

AB-S

5'-GCG GCG ACT AGT GTT CTC TGT AGT CTC TGG GAG AGG-3'

OPR124

5'-GGG GGG AAG CTT GAT ATC TCA GCT TCC AGC AGT AGT ATC AAA
GAA GAA CAT GTG ATC-3'

OPR125

5'-GGG GGG CGG CCG CTA CTT ACC TTT CTC TGG GAG AGG GCT TGG
AGC-3'

DR1B-B

5'-GCG GCG GGA TCC CTT GCT CTG TGC AGA TTC AGA CC-3'

BF-NN

5'-GGG GGG GCC ATG GCC GGA TCC GCT ACC GGG GAC ACC CCA CCA
CGT TTC TTG-3'

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Figure 3 - cont.

BB-5

5'-GCC GCG ACT AGT CTT GCT CTG TGC AGA TTC AGA CCG-3'

OPR121

5'-GTT GTC TTA AGT GGA GCT AGC GGA GGG GGC GGG TCC GGA GGT
GGT GGG GAC ACC CG-3'

OPR122

5'-GAA ATG ACA TTC AAA CTT CAG CTG CCA CAA GAA ACC TGG TCG
GGT GTC CCC ACC ACC-3'

OPR123

5'-GGG GGG CGG CCG TAC CTG AGG ACT TGC TCT GTG CAG ATT CAG-
3'

Peptide oligonucleotides.

Ova 323-332

OPR110

5'-TTA AGT ATC TCT CAG GCT GTT CAC GCT GCT CAC GCT GAA ATC
AAC GAA GCT GGT CGT G-3'

OPR111

5'-CTA GCA CGA CCA GCT TCG TTG ATT TCA GCC TGA GCA GCG TGA
ACA GCC TGA GAG ATA C-3'

Ova H331R

OPR115

5'-TTA AGT ATC TCT CAG GCT GTT CAC GCT GCT CGG GCT GAA ATC
AAC GAA GCT GGT CGT G-3'

OPR116

5'-CTA GCA CGA CCA GCT TCG TTG ATT TCA GCC CGA GCA GCG TGA
ACA GCC TGA GAG ATA C-3'

Ova A332Y

OPR117

5'-TTA AGT ATC TCT CAG GCT GTT CAC GCT GCT CAC TAC GAA ATC
AAC GAA GCT GGT CGT G-3'

OPR116

5'-CTA GCA CGA CCA GCT TCG TTG ATT TCA TAG TGA GCA GCG TGA
ACA GCC TGA GAG ATA C-3'

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Figure 3 - cont.

HEL 74-86

OPR140

5'-TTA AGT AAC CTG TGC AAC ATC CCC TGC AGC GCC CTG CTG AGC
TCC G-3'

OPR141

5'-CTA GCG GAG CTC AGC AGG GCG CTG CAGBGGG ATG TTG CAC AGG
TTA C-3'

NP 404-415

OPR128

5'-TTA AGT CAG ATC AGC GTG CAG CCC GCC TTC AGC GTG CAG G-3'

OPR129

5'-CTA GCC TGC ACG CTG AAG GCG GGC TGA ACG CTG ATC TGA C-3'

HA 307-319

OPR130

5'-TTA AGT CCC AAG TAC GTG AAG CAG AAC ACC CTG AAG CTG GCC
ACC G-3'

OPR131

5'-CTA GCG GTG GCC AGC TTC AGG GTG TTC TGC TTC ACG TAC TTG
GGA C-3'

MBP 91-103

VW315

5'-TTA AGT CAC TAT GGC TCC CTG CCG CAG AAG TCC CAG CAC GGG
CGC G-3'

VW316

5'-CTA GCG CGC CCG TGC TGG GAC TTC TGC GGC AGG GAG CCA TAG
TGA C-3'

PLP 139-151

VW313

5'-TTA CAT CAC TCC CTG GGC AAG TGG CTG GGC CAC CCG GAC AAG
TTC G-3'

VW314

5'-CTA GCG AAC TTG TTC GGG TGG CCC AGC CAC TTG CCC AGG GAG
TGA C-3'

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Figure 8 - cont.

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MBP 1-14

VW317

5' - TTA AGT ATG GCA TCC CAG AAG CGC CCG TCC CAG CGC TCC AAG
TAC CTG G-3'

VW316

5' - CTA GCC AGG TAC TTG GAG CGC TGG GAC GGG CGC TTC TGG GAT
GCC ATA C-3'

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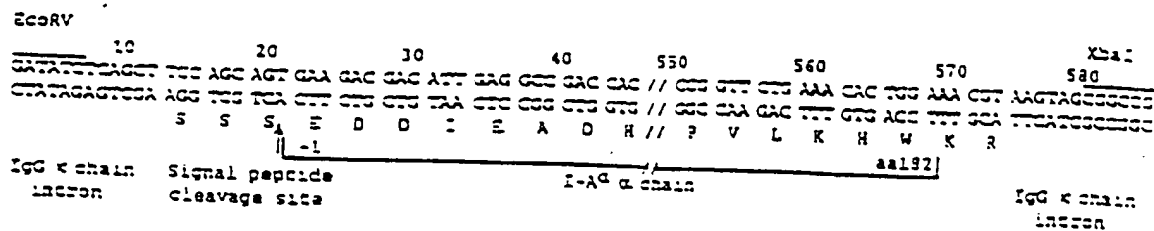
Figure 9A. Soluble I-A^d α chain construct

Figure 9B.

Soluble I-A^d β chain construct

Restriction sites for insertion of
oligonucleotides encoding peptides of interest:

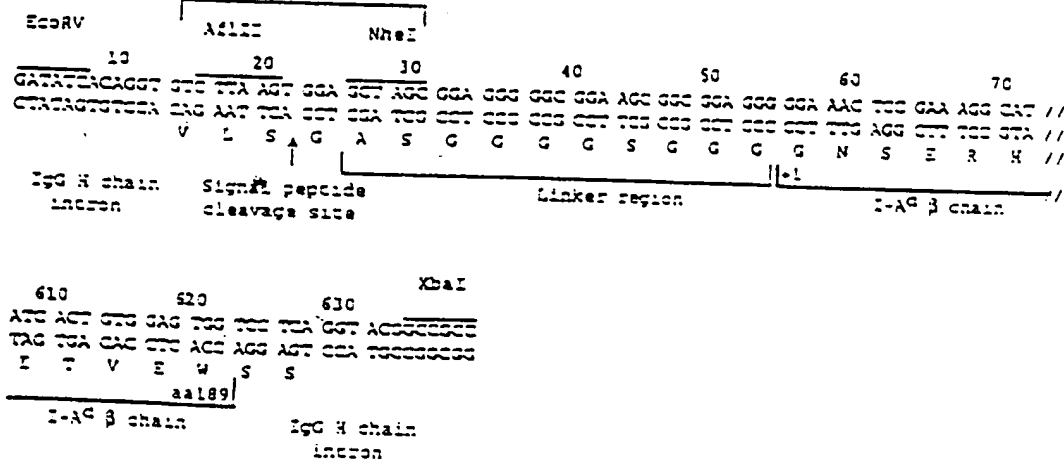


Figure 9C.

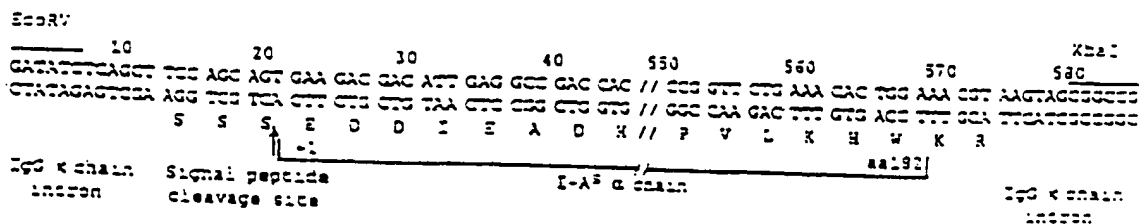
Soluble I-A^s α chain construct

Figure 9 - cont.
Figure 9D. Soluble I-A^s β chain construct

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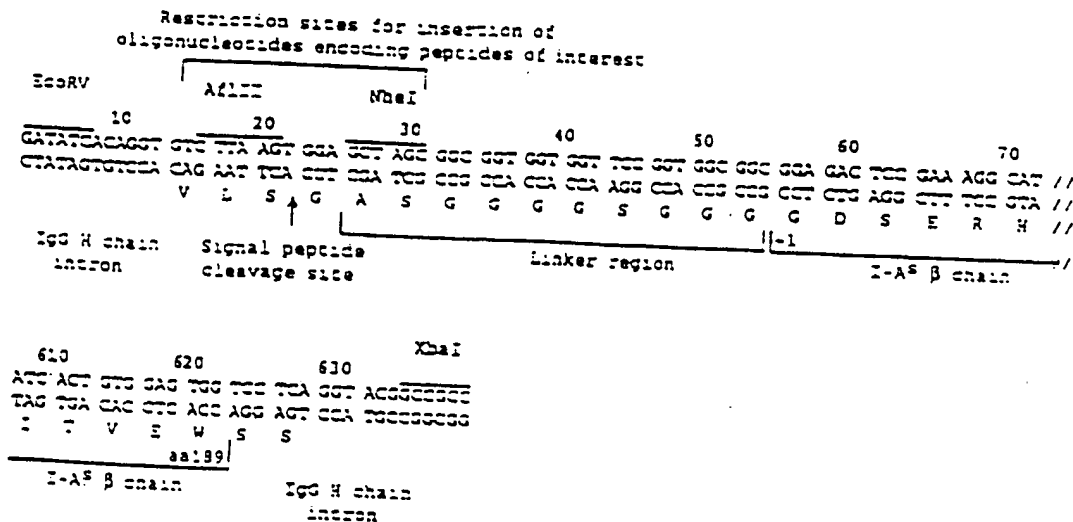


Figure 9E. Soluble HLA-DR1 α chain construct

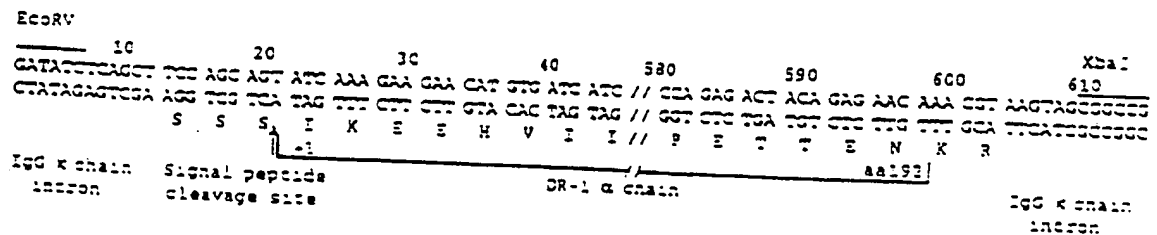
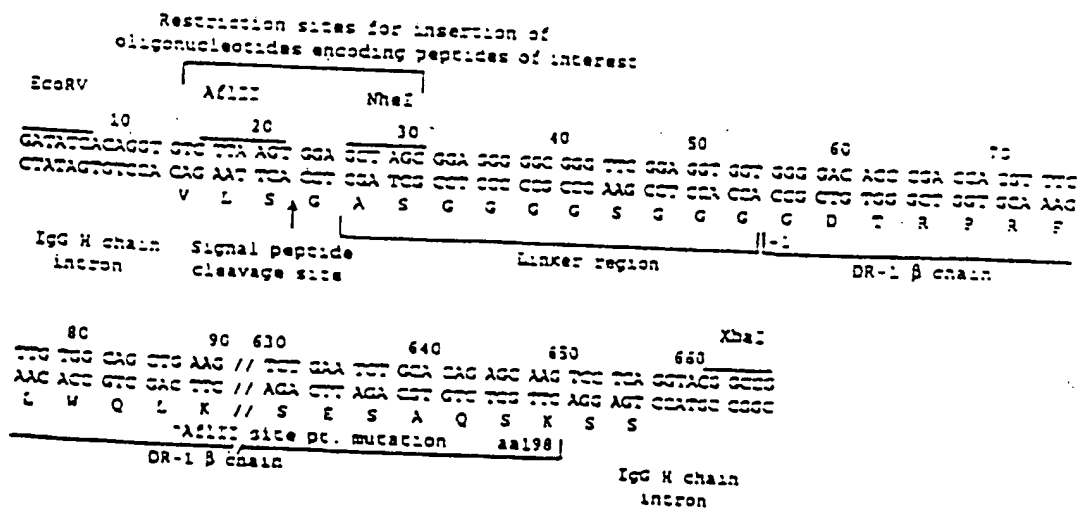
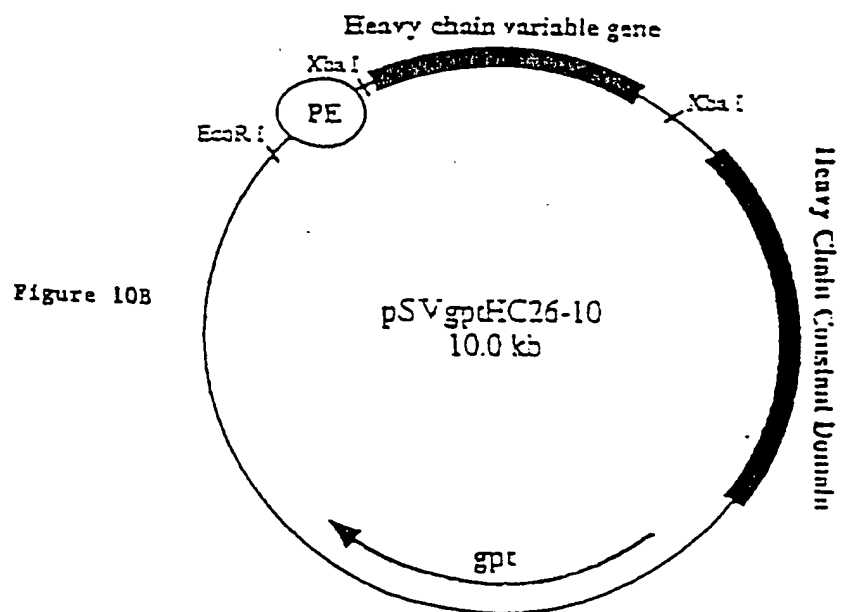
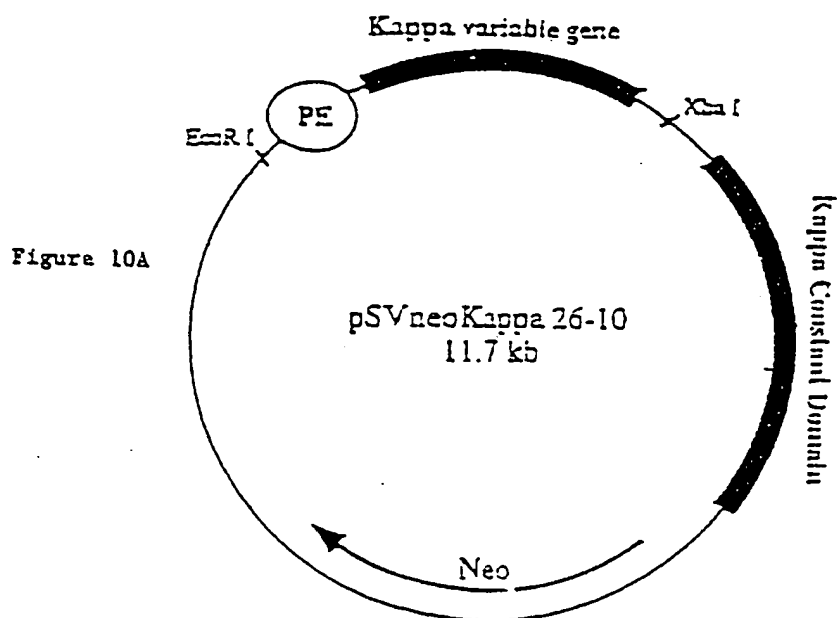


Figure 9F. Soluble HLA-DR1 β chain construct



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Figure 10. Original Mammalian Cell Expression Vectors



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Figure 11. The 27 KB Kappa and the 17 Kb Heavy Chain EcoR V and Eag I Mutated Constructs

Figure 11A

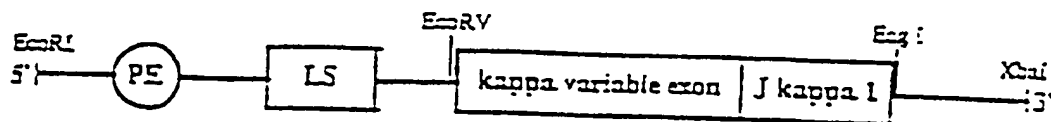
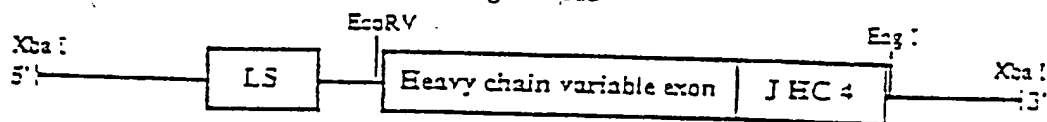
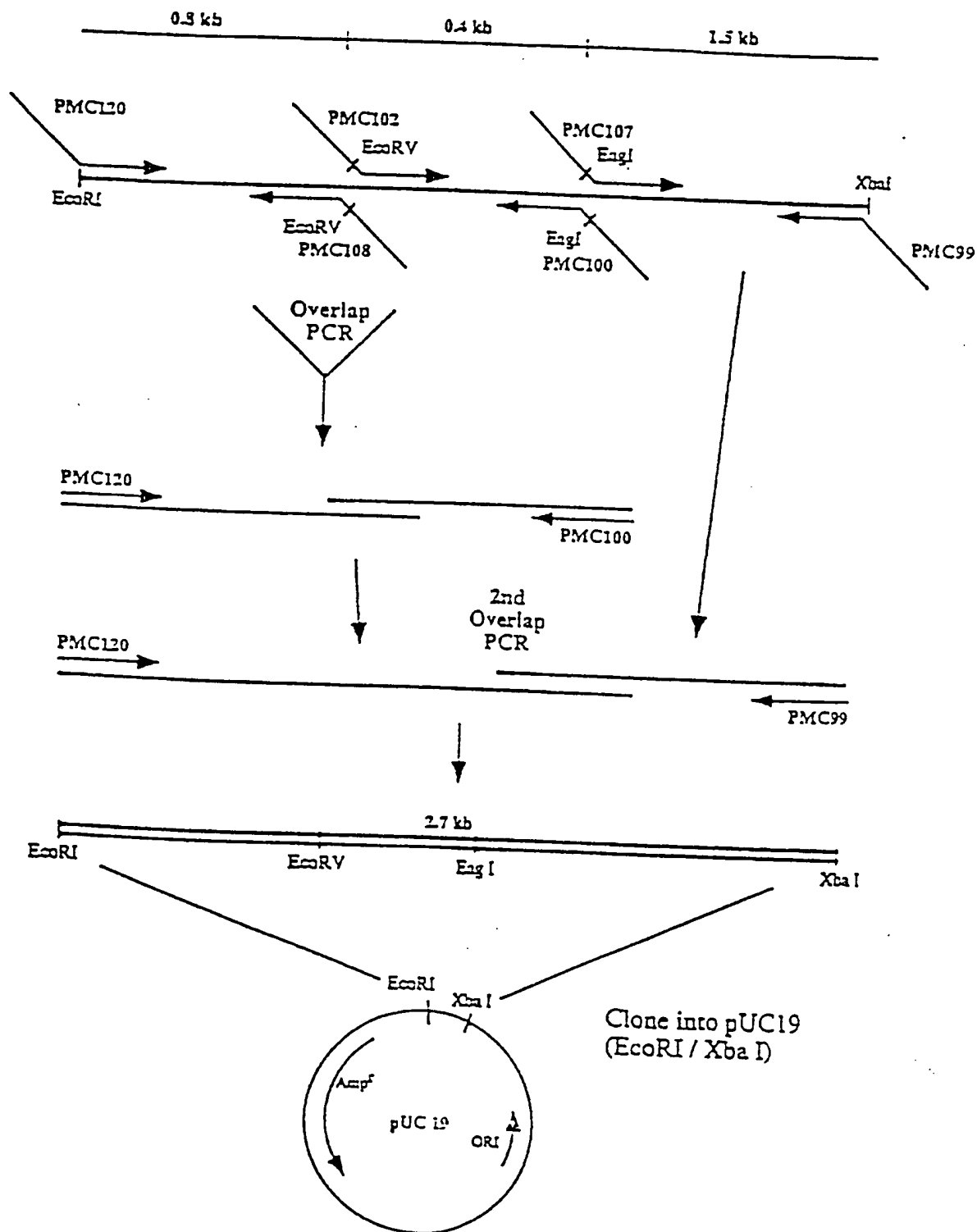


Figure 11B

[illegible]

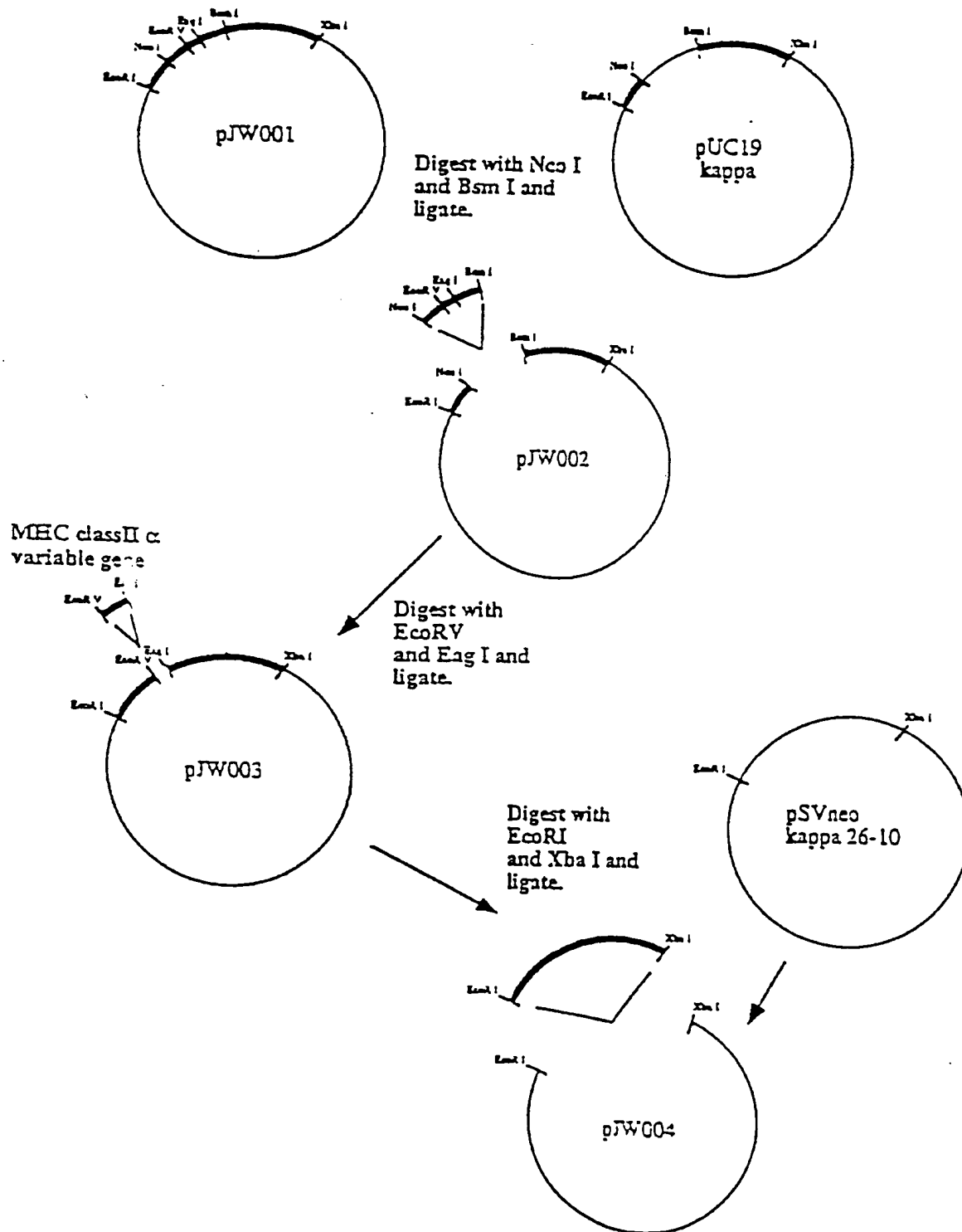
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Figure 12. PCR Site Directed Mutagenesis for Introducing EcoRV and EagI Restriction Sites into Kappa Chain 2.7kb Insert



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Figure 13. STRATEGY FOR CONSTRUCTING MEC CLASS II α / KAPPA CONSTANT GENE IN MAMMALIAN CELL EXPRESSION VECTOR



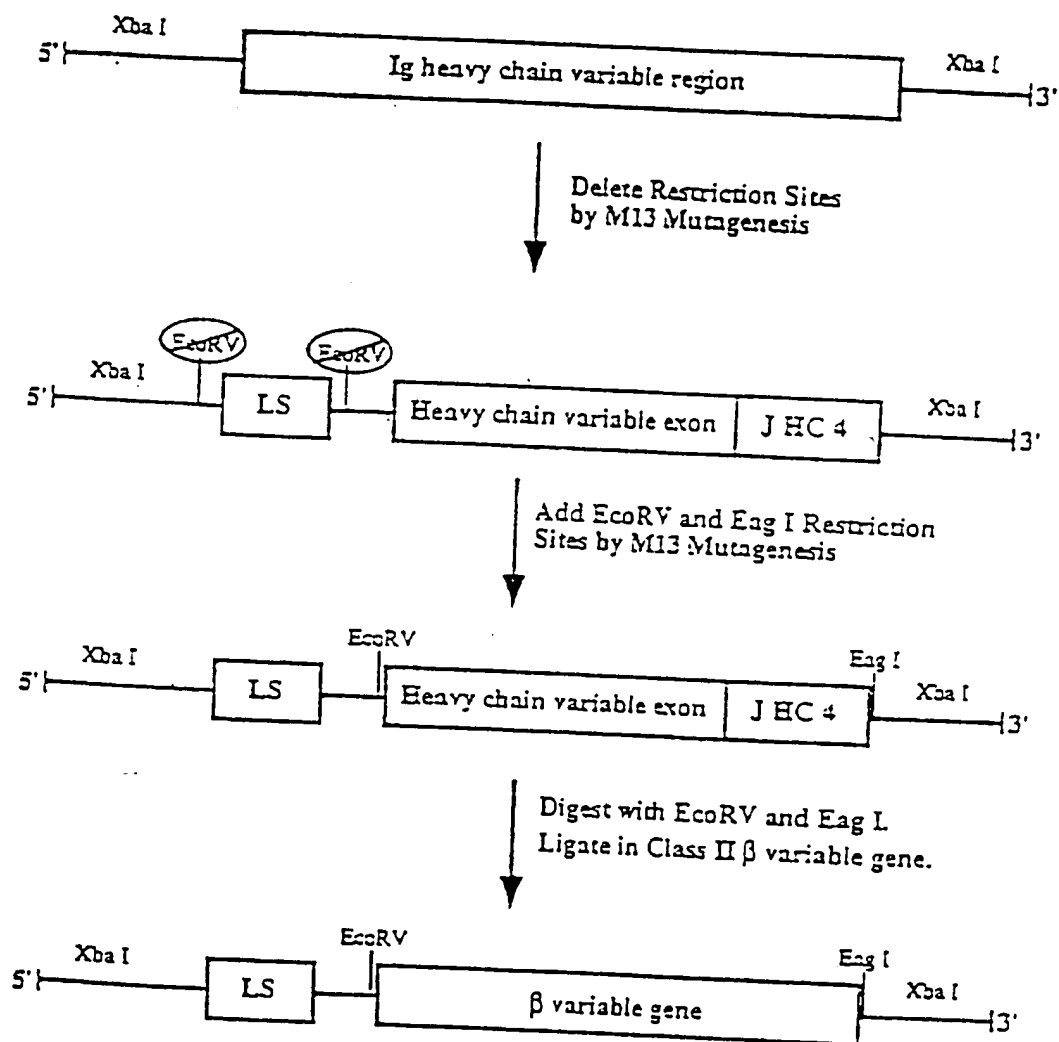
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Figure 14. Primers used for Sequencing Mutated 2.7 Kb Fragment

<u>Primer List</u>	<u>Sequence</u>
PMC-33	[5GCTCAGCTGTCTTGTTTCAGTACTGATG3']
PMC-77	[5GTAAGTAGCGGCCG3']
PMC-111	[5GGTATGTAAAAATAAACATCACAG3']
PMC-114	[5GCTTTGCTTACGGAGTTACTG3']

FOIA b2 b7D b7E b7F b7G b7H b7I b7J b7K b7L b7M b7N b7O b7P b7Q b7R b7S b7T b7U b7V b7W b7X b7Y b7Z

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Figure 15. Strategy for M13 Mutagenesis and Cloning of the MEC II β Variable Gene

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Figure 16A

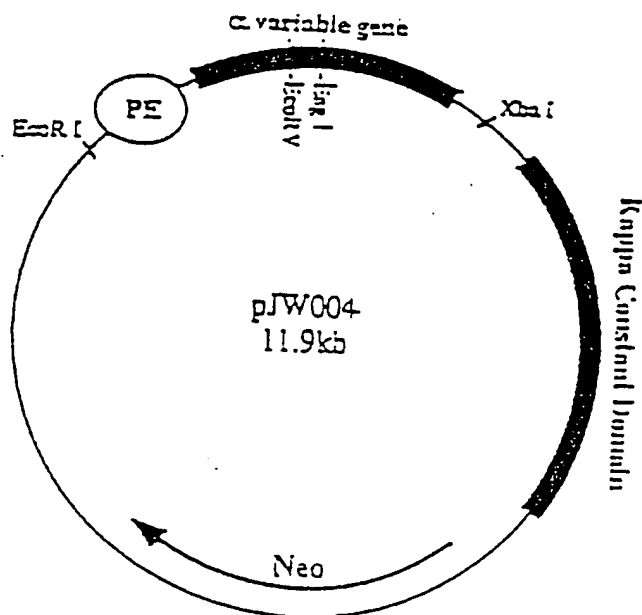
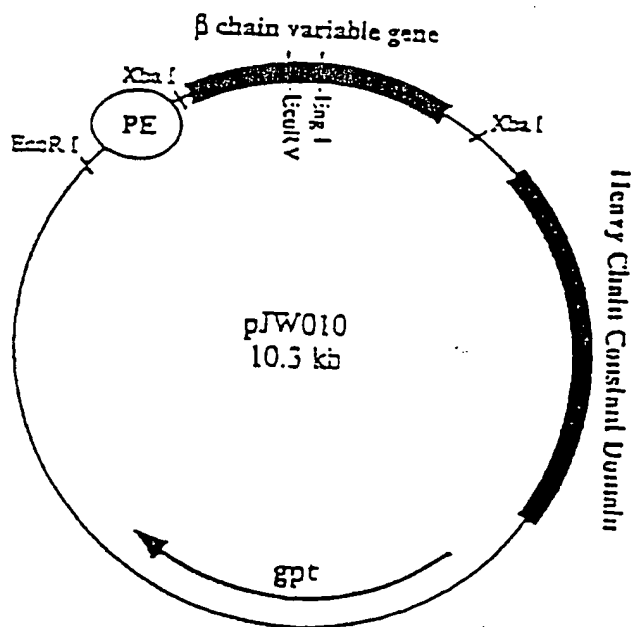
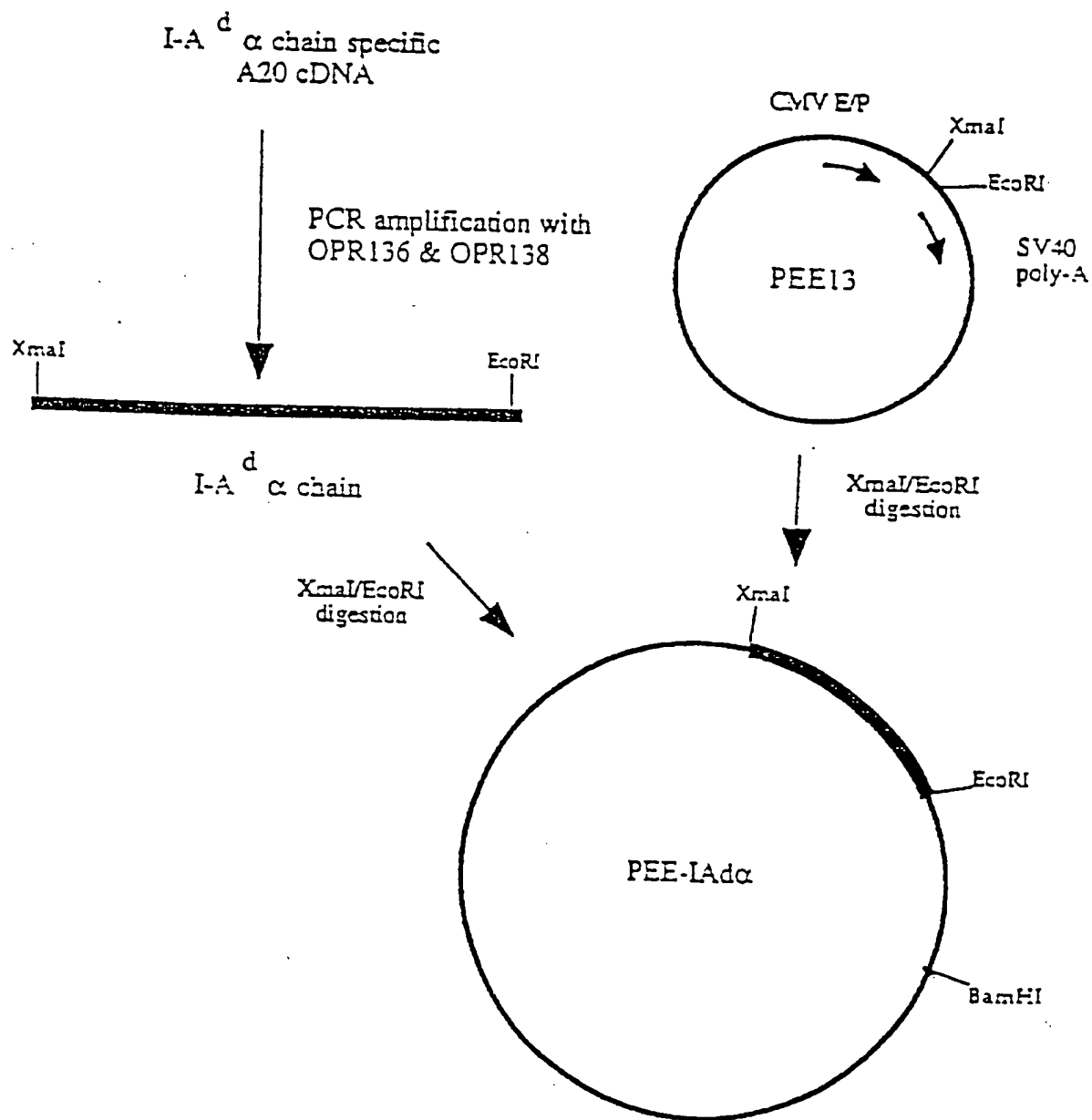


Figure 16B

[illegible]

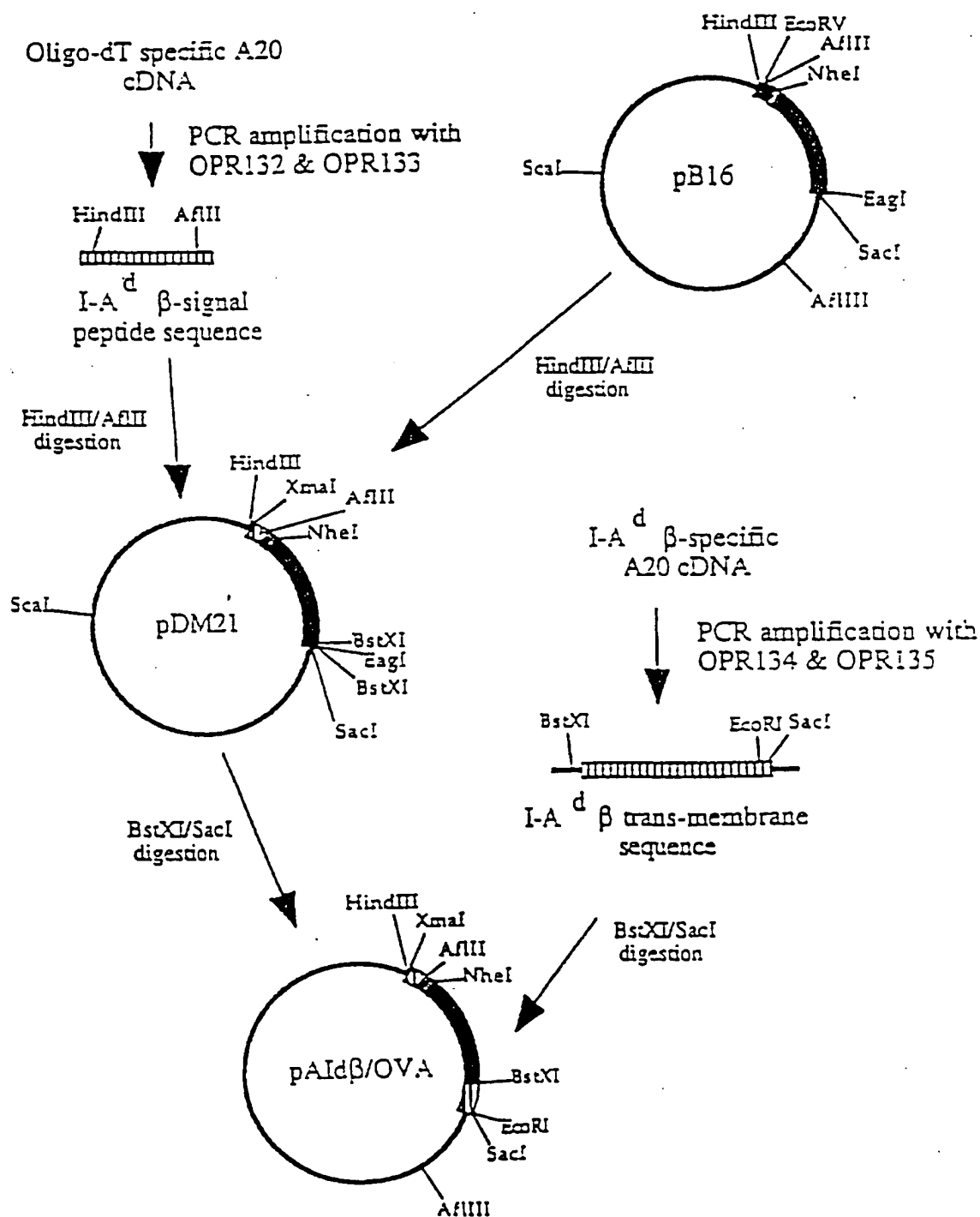
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Figure 17 - Full length peptide linked MHC expression vectors



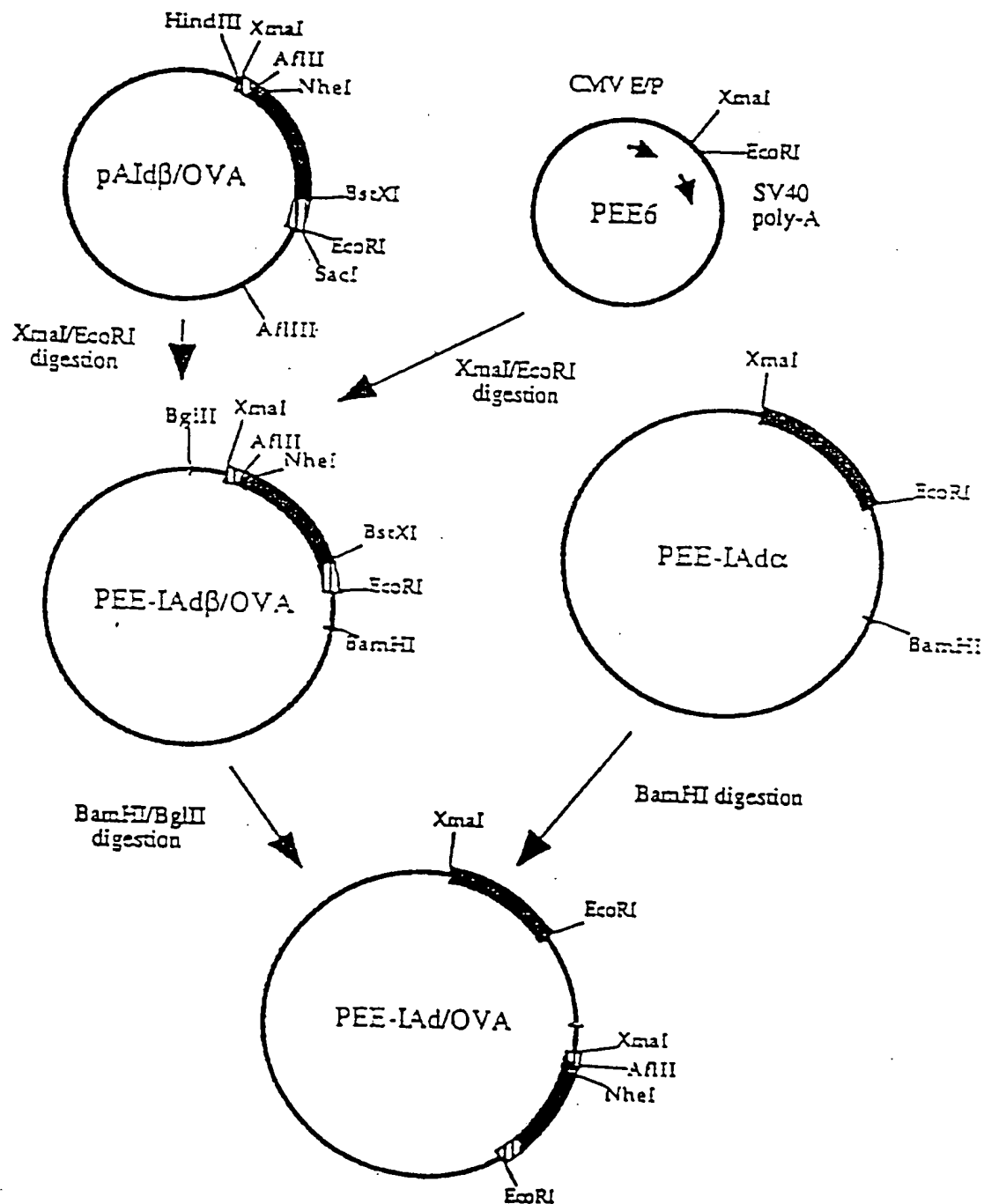
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Figure 17 - cont.



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Figure 17 - cont.



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[illegible]

HindIII XbaI

10 20 30 40 50

AAGCTTCTGCTG GGGGACG ATG GGT GTG CAG ATT TTT AGC GTT GTG GTG TGA GGT
TTTGAAGGCG CCGTTTC CAC CCA CAC GTC TAC GCG TTT CAG GAG GAG AGT GCA
H A L Q I P S L L L S A

Xorak
consensus

I-Ad β chain signal peptide

60 70 80 90 100

GGT GTG GTG GTG GTG ATG GTG GTG AGC AGC CCA AGC AGC TTA AGT ATT
CCA CAC CAC CAC CAC CAC CAC CAC TTT TTT GGT TTT TTT AAT TCA TAG
A V V V L H V L S S P R T L SA I

I-Ad β chain signal peptide

Signal peptide
cleavage site

110 120 130 140

TTT CAG GGT GTT CAC GGT GGT CAC GGT GAA ATT AAC GAA GGT GGT GGT
ACA GTT CCA CCA GTT CCA CCA GTT CCA GGT CAG TTT GTT GTT CCA CCA
S Q A V H A A H A E I N E A G R

Ova peptide

NheI

150 160 170 180 190

GGT AGC CCA GCG GCG CCA AGC GCG CCA GCG CCA AAC TTT GAA AGC //
CCA TTT GGT GGT GGT GGT TTT GGT GGT GGT GGT TTT AGG GTT TTT //
A S G G G G S G G G G N S E R //

Linker region

I-Ad β chain

870 880 890 900

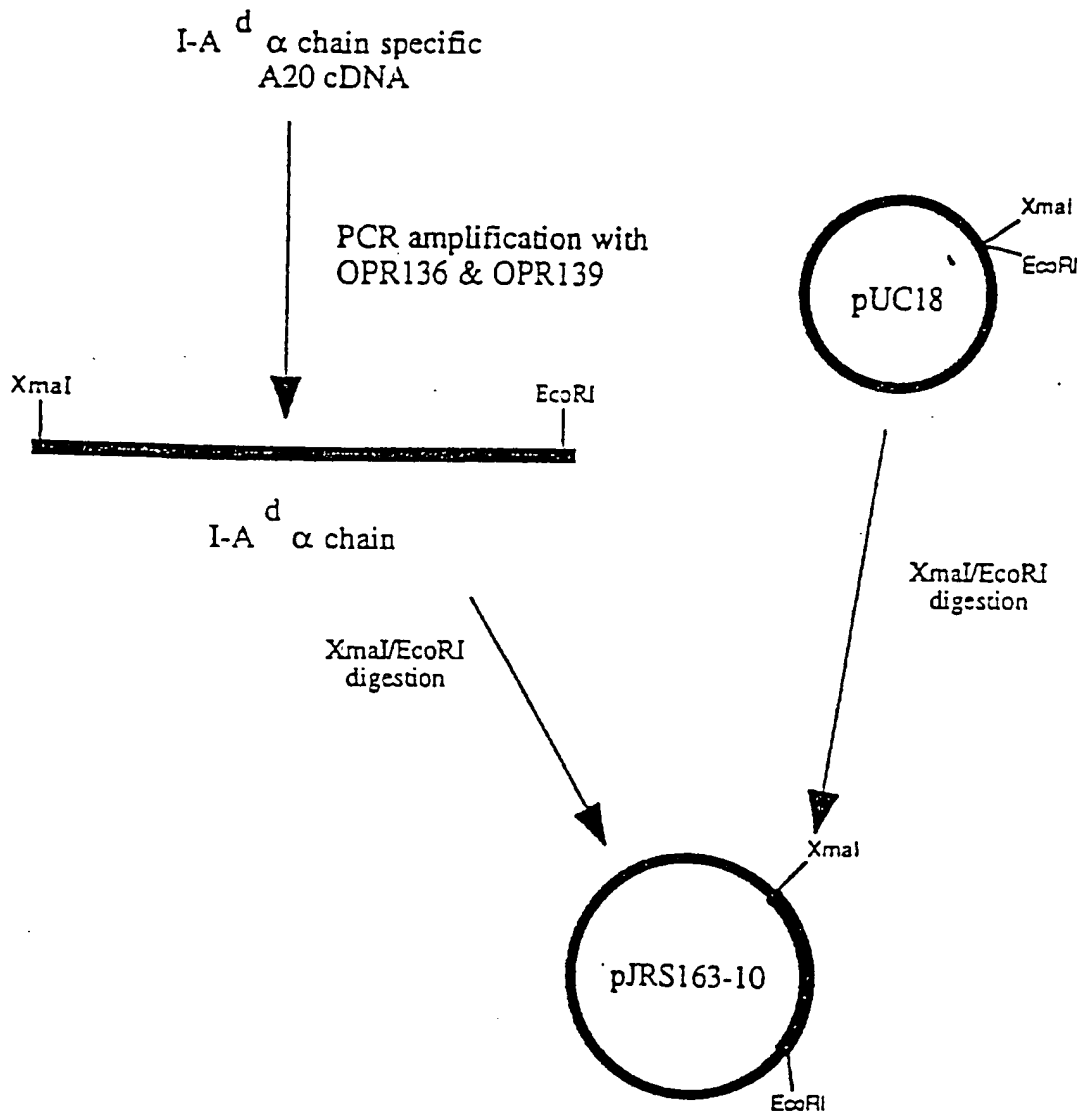
ECOR1 SacI

GGT GGT CCA CCA GCG TTT GGT CAG TCA GAAT TGAAGTT
CCA CCA GGT GGT GGT CAG CAG GTC ACT GTTA AGCTTAC
P P P A G L L Q

I-Ad β chain

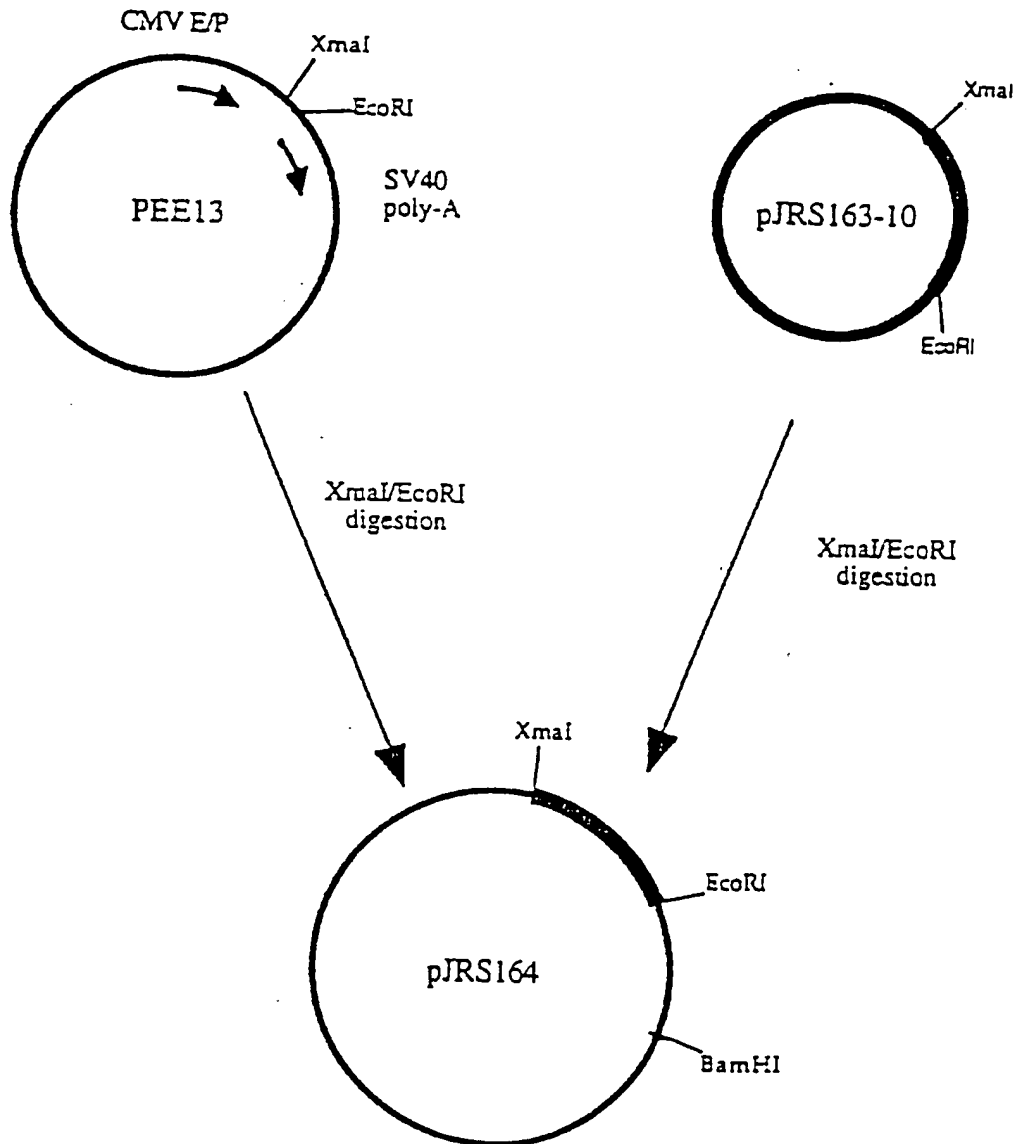
FIGURE 19 (Sheet 1 of 7)

Full-length peptide linked MHC expression vectors



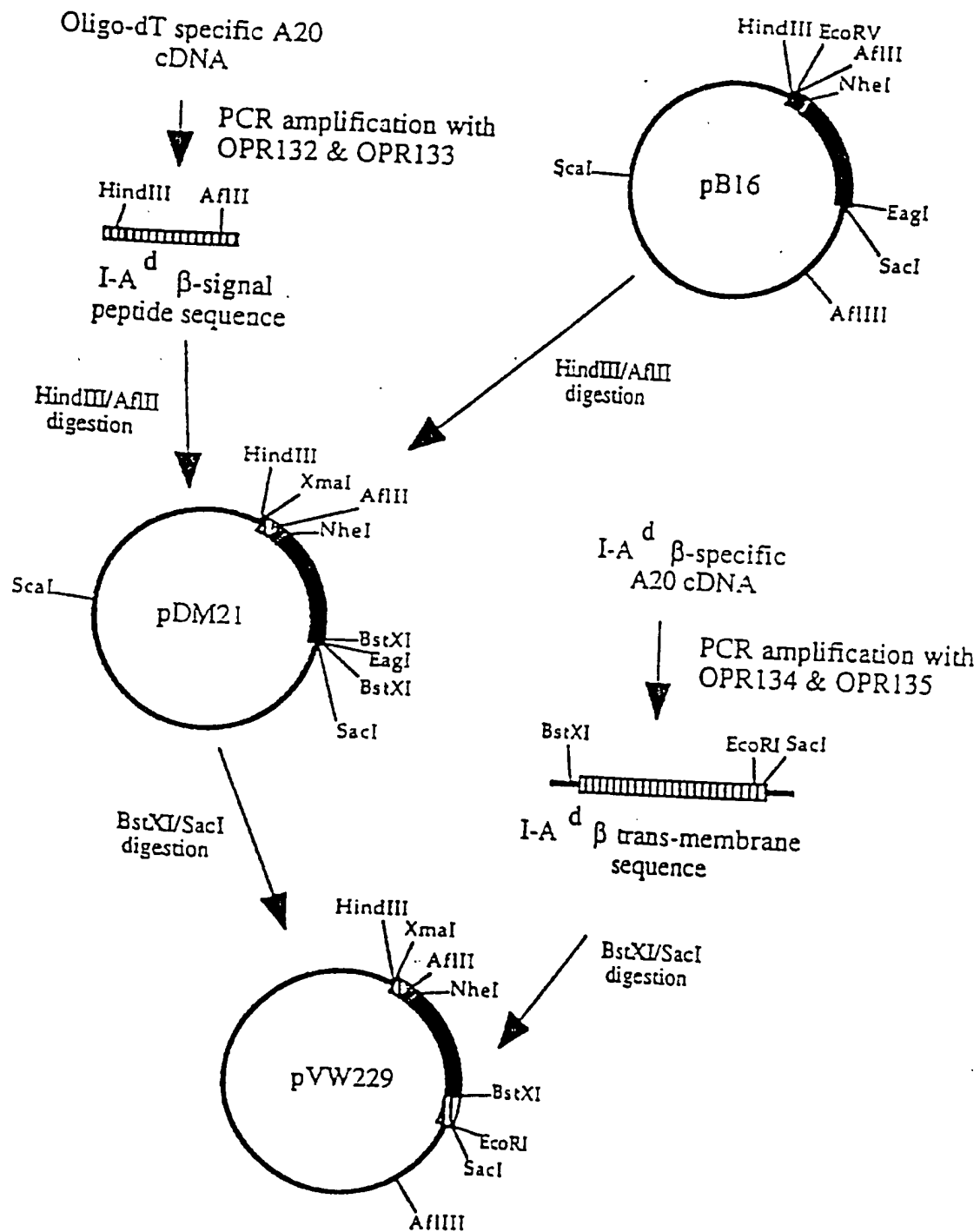
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FIGURE 19 (Sheet 2 of 7)



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FIGURE 19 (Sheet 3 of 7)



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FIGURE 19 (Sheet 4 of 7)

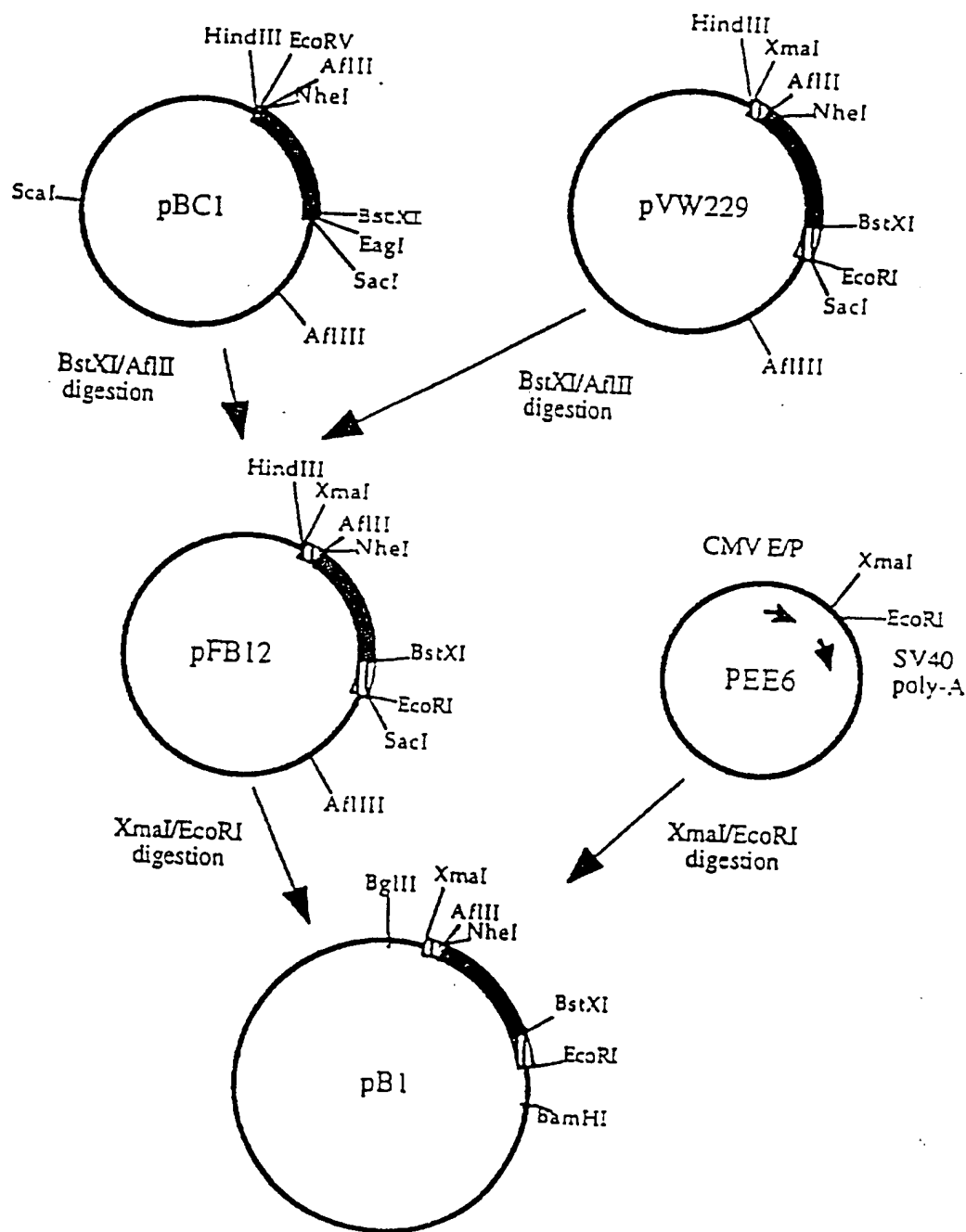
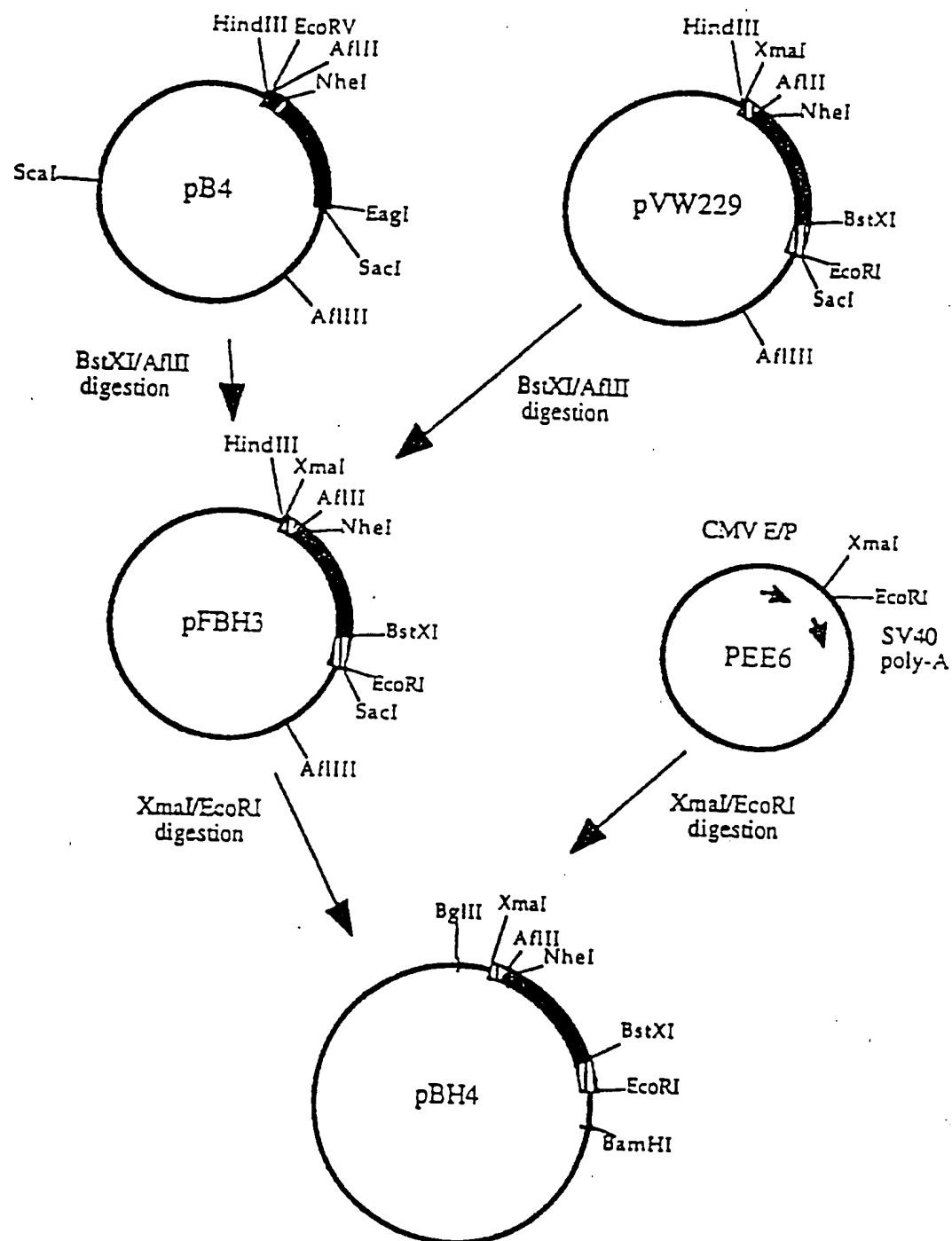
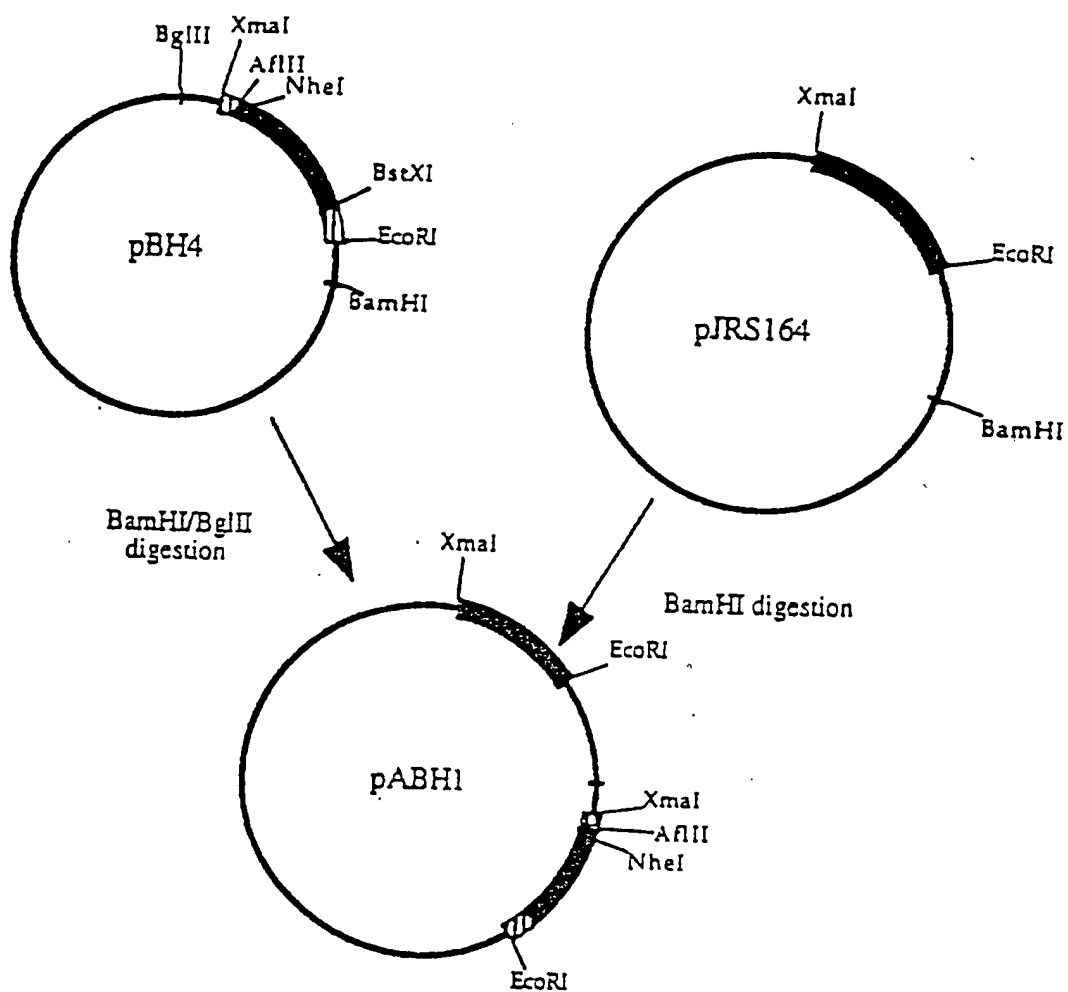


FIGURE 19 (Sheet 4 of 7)

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FIGURE 19 (Sheet 6 of 7)

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FIGURE 19 (Sheet 7 of 7)



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FIGURE 20

Oligonucleotides used in cloning

OPR132

I-A^d β signal peptide front primer with Kozak consensus for CellTech vector -
HindIII/XmaI sites

5'-CCC CCC AAG CTT CCC GGG CCA CCA TGG CTC TGC AGA TCC CCA
GC-3'

OPR133

I-A^d β signal peptide back primer with Kozak consensus for CellTech vector - AflII site

5'-CCC CCC ACT TAA GGT CCT TGG GCT GCT CAG CAC C-3'

OPR134

I-A^d β transmembrane front primer for CellTech vector - BstXI sites

5'-CCC CCC CCA TCA CTG TGG AGT GGA GGG-3'

OPR135

I-A^d β transmembrane back primer for CellTech vector - SstI, EcoRI sites

5'-CCC CCC GAG CTC GAA TCC TCA CTG CAG GAG CCC TGC TGG-3'

OPR136

I-A^d α signal peptide front primer with Kozak consensus for CellTech vector -
HindIII/XmaI sites

5'-CCC CCC AAG CTT CCC GGG CCA CCA TGC CGT GCA GCA GAG CTC
TG-3'

OPR139

I-A^d α transmembrane primer for CellTech vector - SstI/EcoRI sites

5'-CCC CCC GAG CTC GAA TCC TCA TAA AGG CCC TGG GTG TCT G-3'

B7-1-2F

Murine B7-1 front primer with Kozak consensus for CloneTech vector - NotI site

5'-CCC CCC CCG CGG CCG CCC CAC CAT GGG ACT GAG TAA CAT TCT
C-3'

B7-1-2B

Murine B7-1 BACK primer for CloneTech vector - NotI site

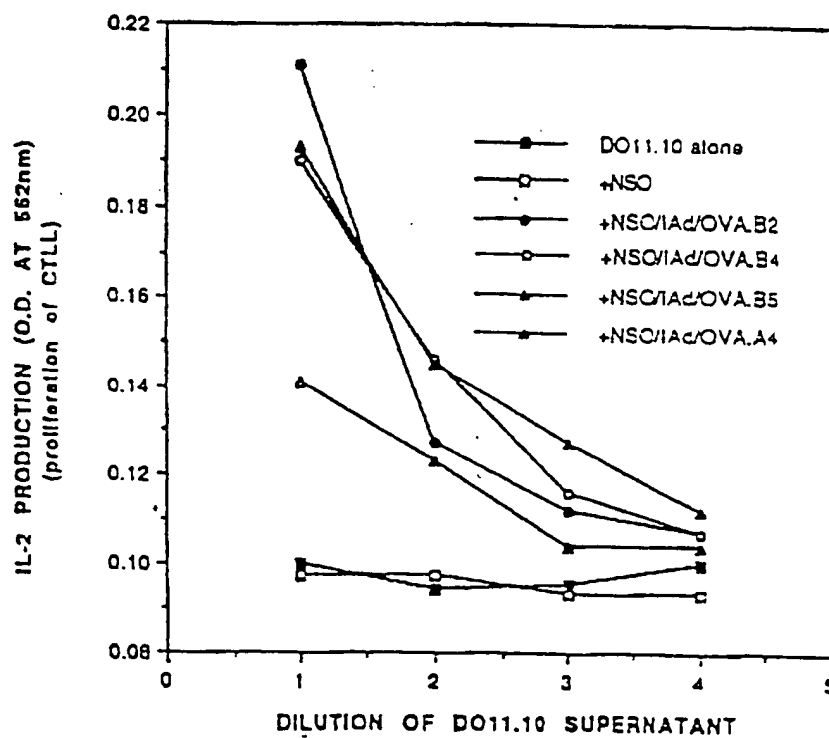
5'-CCC CCC GCG GCC GCT TTA AAA ACA TGT ATC ACT TTT-3'

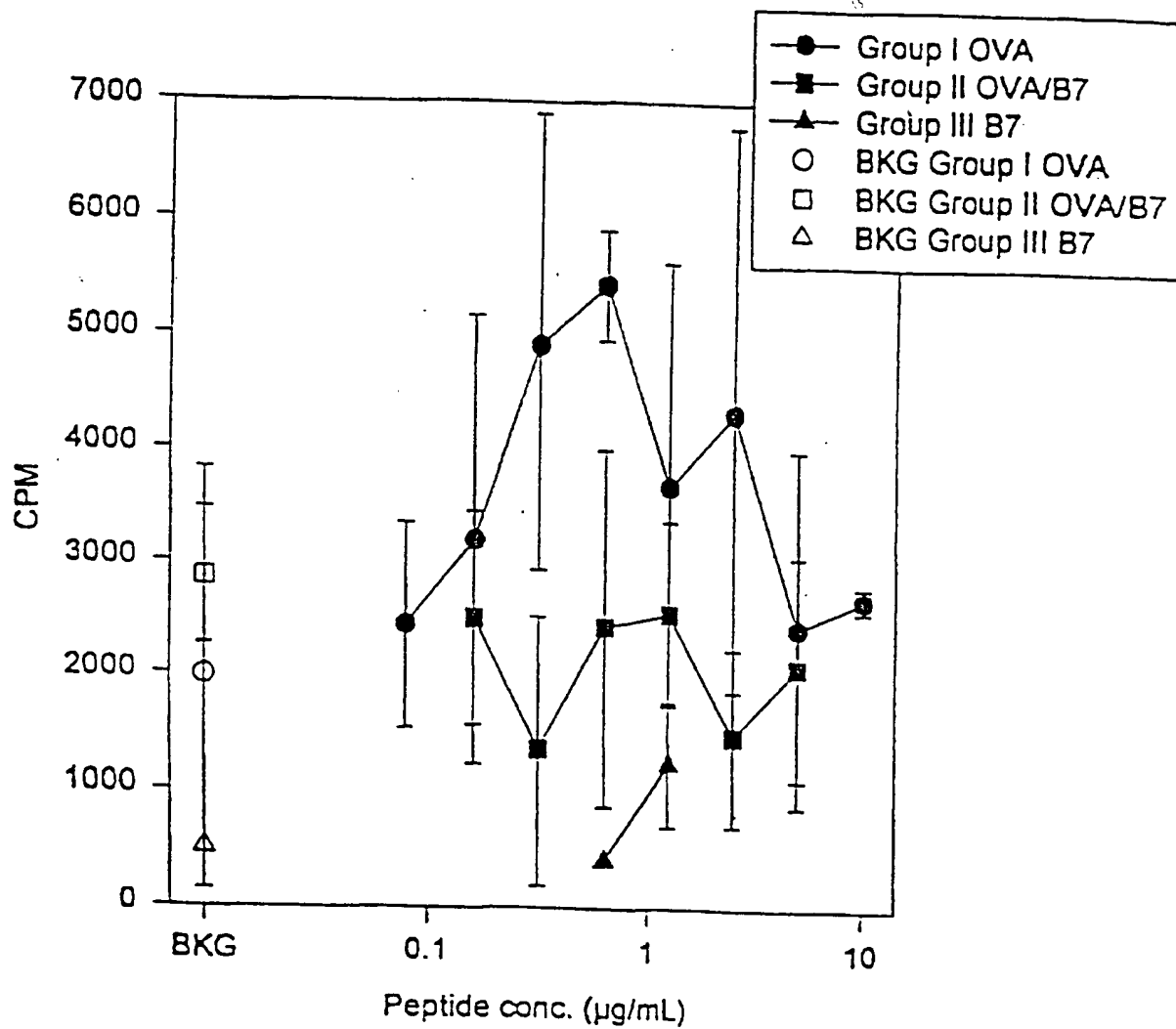
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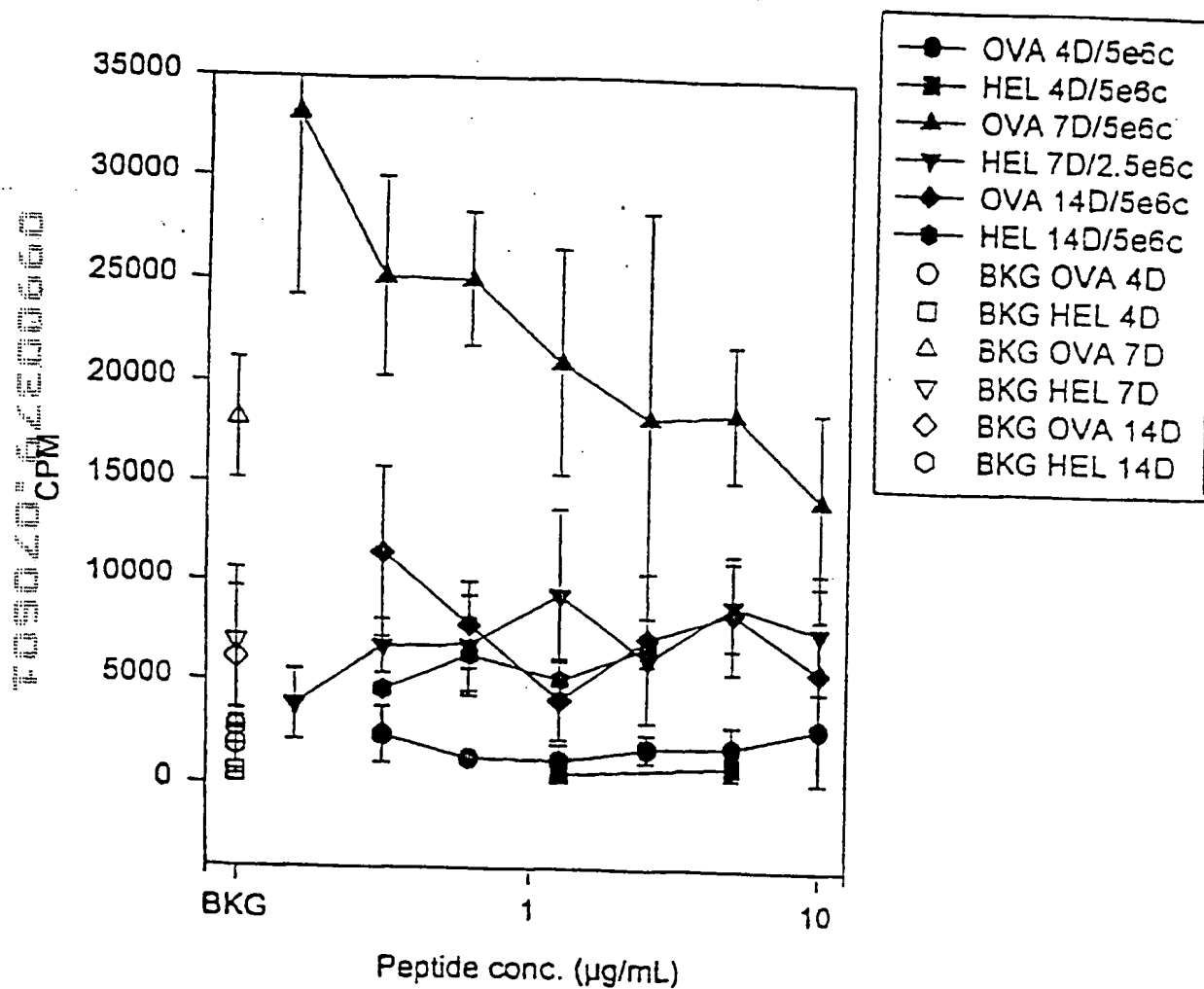
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FIGURE 21

NSO/ClassII/OVA clones Stimulate IL-2 Production from DO11.10

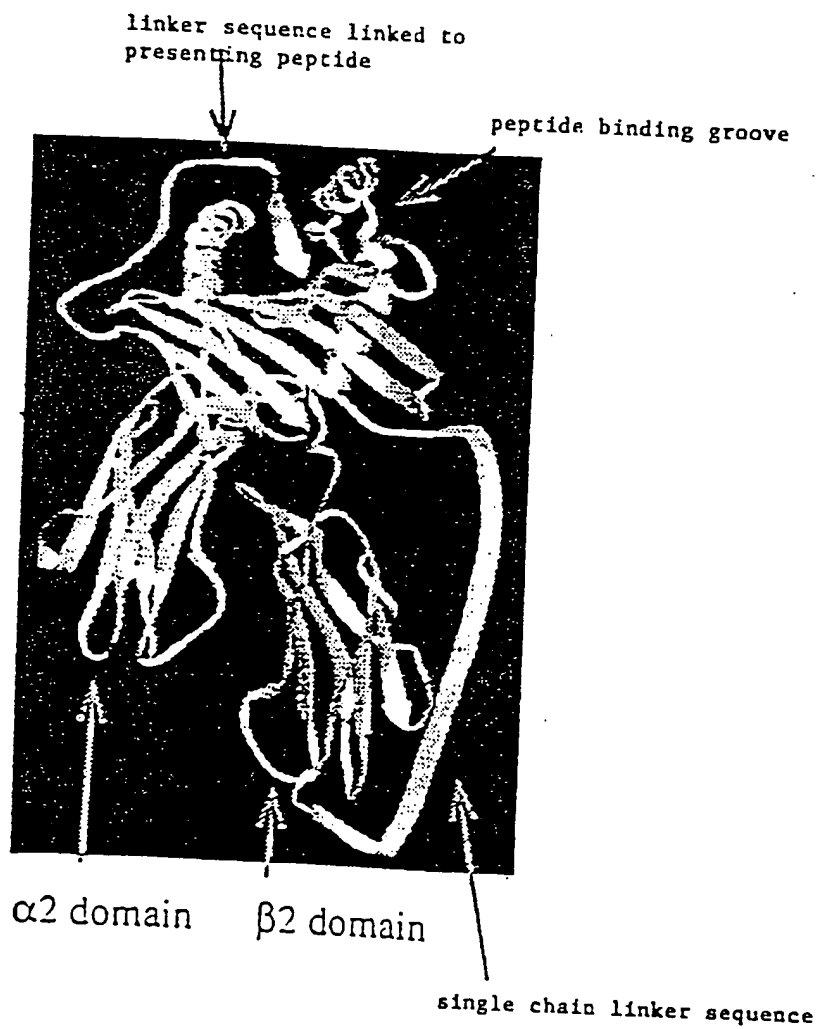


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FIGURE 22INTRAMUSCULAR IAd/OVA & B7 DNA INJECTIONS
PROLIFERATION ASSAY

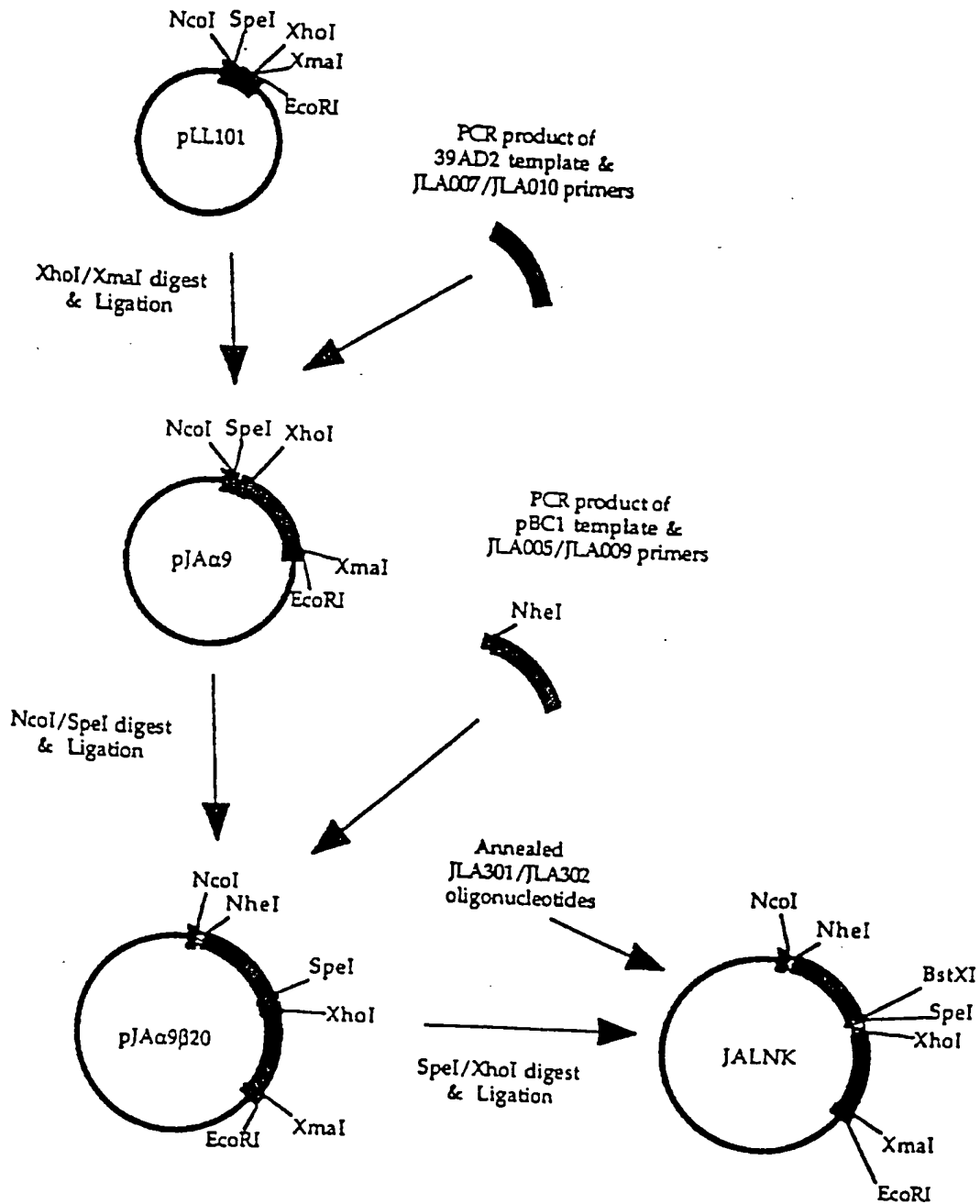
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FIGURE 23**INTRADERMAL IAd/OVA & IAd/HEL DNA INJECTIONS
PROLIFERATION ASSAY
4, 7 & 14 DAYS POST INJECTION**

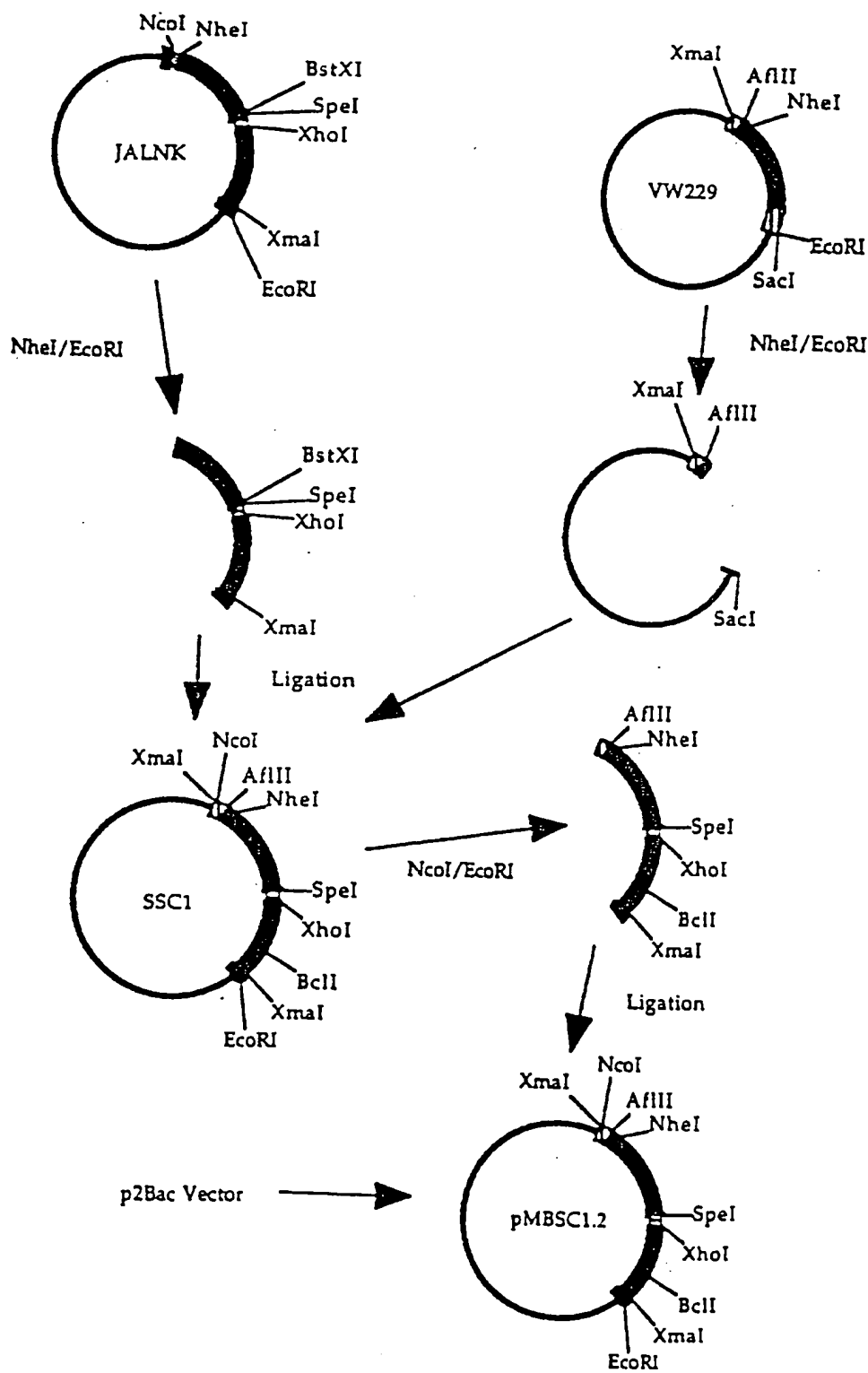
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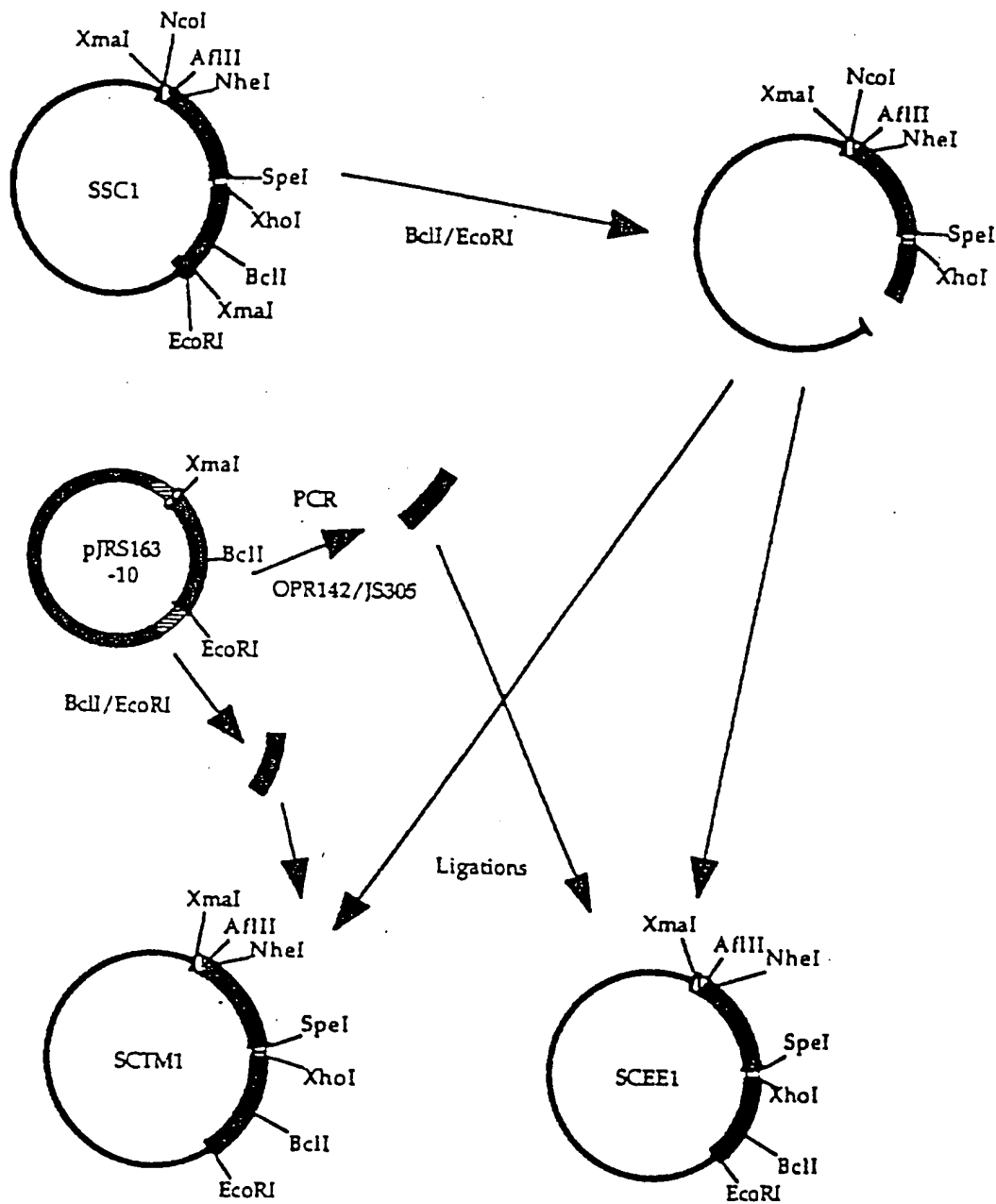
FIGURE 24



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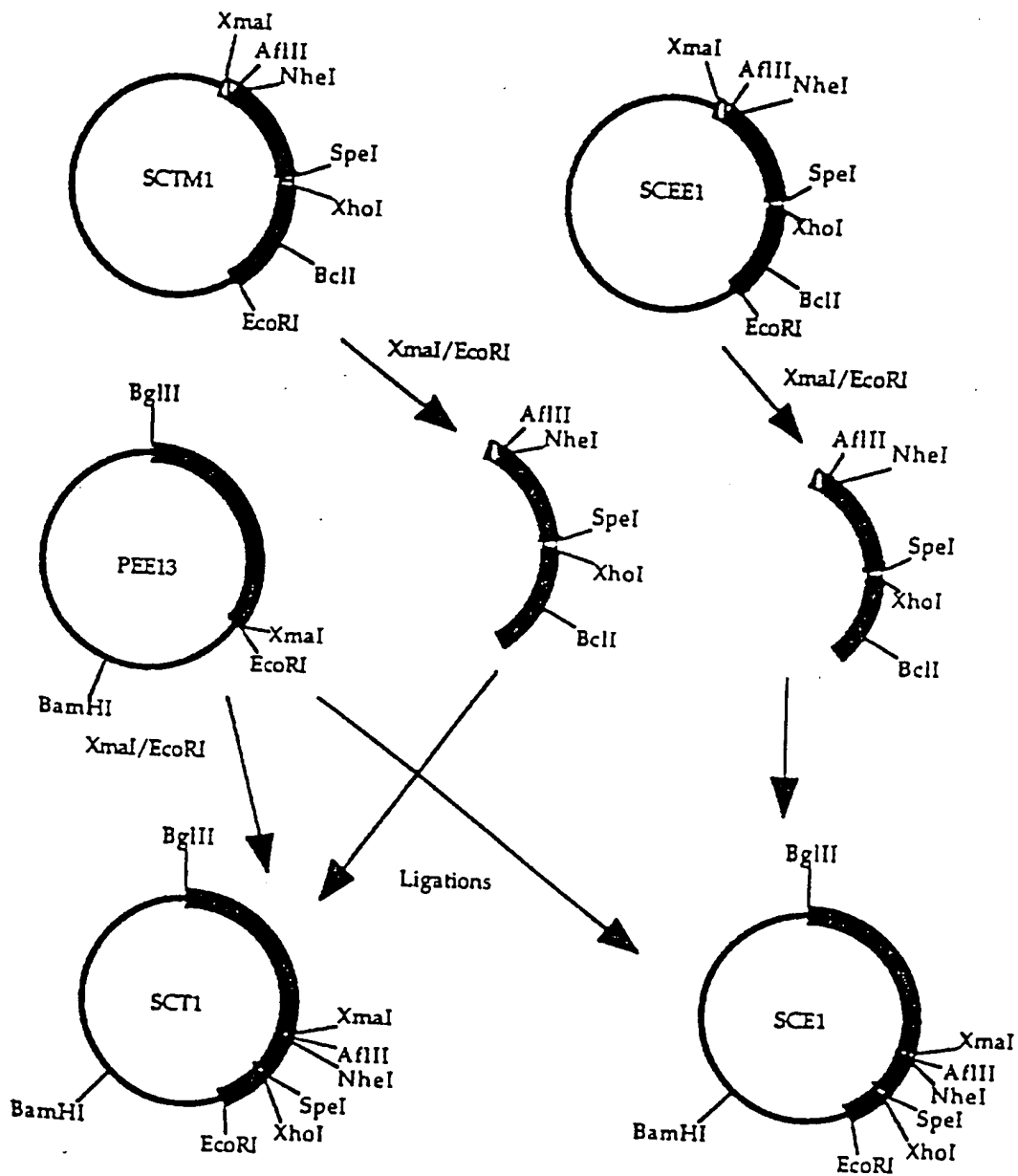
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FIGURE 25 (SHEET 1 OF 4)

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FIGURE 25 (SHEET 2 OF 4)

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FIGURE 25 (SHEET 3 OF 4)

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FIGURE 25 (SHEET 4 OF 4)



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FIGURE 26

JLA-005

5'-CCCCCGCCATGGCCGCTAGCGGAGGGGGCGGAAGC-3'

JLA-007

5'-CCCGGGGCTCGAGTGAAGACGACATTGAGGCCGAC-3'

JLA-009

5'-CCCCCACTAGTCCACTCCACAGTGATGGGGCT-3'

JLA-010

5'-CCCCCCCCCGGGACCAGTGTTTCAGAACCGGCTCCTC-3'

JLA-301

5'-TCGAGGAACCGCCACCGCCAGAACCGCCGCCACCGGA-
ACCACCACCGCCGCTGCCACCGCCACCA-3'

JLA-302

5'-CTAGTGGTGGCGGTGGCAGCGGCGGTGGTGGTTCCGG-
TGGCGGCGGTTCTGGCGGTGGCGGTTC-3'

OPR-142

5'-CTTGGAATCTTGACTAAGAGG-3'

JS-305

5'-CAGGTCTGAATTCTCATTCCATCGGCATGTACTCTTCTT-
CCTCCCAGTGTTTCAGAACCGG-3'

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FIGURE 27 (SHEET 1 OF 4)

```

      10      20      30      40      50
      .      .      .      .      .
CCACC ATG GCT CTG CAG ATC CCC AGC CTC CTC TCA GCT GCT GTG GTG
GGTGG TAC CGA GAC GTC TAG GGG TCG GAG GAG GAG AGT CGA CGA CAC CAC
      M   A   L   Q   I   P   S   L   L   L   S   A   A   V   V>
<----- I-Ad β chain leader ----->

      60      70      80      90
      .      .      .      .
GTG CTG ATG GTG CTG AGC AGC CCA AGG ACC TTA AGT ATC TCT CAG GCT
CAC GAC TAC CAC GAC TCG TCG GGT TCG AAT TCA TAG AGA GTC CGA
      V   L   M   V   L   S   S   P   R   T   L   S   I   S   Q   A>
----->-----<-----

    100      110      120      130      140
    .      .      .      .      .
GTT CAC GCT GCT CAC GCT GAA ATC AAC GAA GCT GGT CGT GCT AGC GGA
CAA GTG CGA CGA GTG CGA CTT TAG TTG CTT CGA CCA GCA CGA TCG CCT
      V   H   A   A   H   A   E   I   N   E   A   G   R   A   S   G>
----- OVA 323-339 ----->-----<-----

    150      160      170      180      190
    .      .      .      .      .
GGG GGC GGA AGC GGC GGA GGG CGA AAC TCC GAA AGG CAT TTC GTG GTC
CCC CCG CCT TCG CCG CCT CCC CCT TTG AGG CTT TCC GTA AAG CAC CAG
      G   G   G   S   G   G   G   G   N   S   E   R   H   F   V   V>
-- 10 amino acid linker -->----- I-Ad β-1 domain -----<-----

    200      210      220      230      240
    .      .      .      .      .
CAG TTC AAG GGC GAG TGC TAC TAC ACC AAC GGG ACG CAG CGC ATA CGG
GTC AAG TTC CCG CTC ACG ATG ATG TCG TTG CCC TCG GTC GCG TAT GCC
      Q   F   K   G   E   C   Y   Y   T   N   G   T   Q   R   I   R>
----->-----<-----

    250      260      270      280      290
    .      .      .      .      .
CTC GTG ACC AGA TAC ATC TAC AAC CGG GAG GAG TAC GTG CGC TAC GAC
GAG CAC TCG TCT ATG TAG ATG TTG GCC CTC CTC ATG CAC GCG ATG CTG
      L   V   T   R   Y   I   Y   N   R   E   E   Y   V   R   Y   D>
----->-----<-----

    300      310      320      330
    .      .      .      .
AGC GAC GTG GGC GAG TAC CGC GCG GTG ACC GAG CTG GCG CGG CCA GAC
TCG CTG CAC CCG CTC ATG GCG CGC CAC TCG CTC GAC CCC GCC GGT CTG
      S   D   V   G   E   Y   R   A   V   T   E   L   G   R   P   D>
----->-----<-----

    340      350      360      370      380
    .      .      .      .      .
GCC GAG TAC TCG AAC AGC CAG CCG GAG ATC CTG GAG CGA ACG CGG GCC
CGG CTC ATG ACC TTG TCG GTC GCG CTC TAG GAC CTC GCT TCG GCC CGG
      A   E   Y   W   N   S   Q   P   E   I   L   E   R   T   R   A>
----->-----<-----

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FIGURE 27 (SHEET 2 OF 4)

```

390      400      410      420      430
GAG GTG GAC ACG GCG TGC AGA CAC AAC TAC GAG GGG CCG GAG ACC AGC
CTC CAC CTG TGC CGC ACG TCT GTG TTG ATG CTC CCC GGC CTC TGG TCG
E V D T A C R H N Y E G P E T S>
-----

440      450      460      470      480
ACC TCC CTG CCG CGG CTT GAA CAG CCC AAT GTC GCC ATC TCC CTG TCC
TGG AGG GAC GCC GCC GAA CTT GTC GCG TTA CAG CGG TAG AGG GAC AGG
T S L R R L E Q P N V A I S L S>
--- I-Ad β-1 domain ->----- I-Ad β-2 domain -----

490      500      510      520      530
AGG ACA GAG GCC CTC AAC CAC CAC AAC ACT CTG GTC TGT TCG GTG ACA
TCC TGT CTC CGG GAG TTG GTG TTG TGA GAC CAG ACA AGC CAC TGT
R T E A L N H H N T L V C S V T>
-----

540      550      560      570
GAT TTC TAC CCA GCC AAG ATC AAA GTG CGC TGG TTC AGG AAT GGC CAG
CTA AAG ATG GGT CGG TTC TAG TTT CAC GCG ACC AAG TCC TTA CCG GTC
D F Y P A K I K V R W F R N G Q>
-----

580      590      600      610      620
GAG GAG ACA GTG GGG GTC TCA TCC ACA CAG CTT ATT AGG AAT GGG GAC
CTC CTC TGT CAC CCC CAG AGT AGG TGT GTC GAA TAA TCC TTA CCC CTG
E E T V G V S S T Q L I R N G D>
-----

630      640      650      660      670
TGG ACC TTC CAG GTC CTG GTC ATG CTG GAC ATG ACC CCT CAT CAG GGA
ACC TGG AAG GTC CAG GAC CAG TAC GAC CTC TAC TGG GGA GTA GTC CCT
W T F Q V L V M L E M T P H Q G>
-----

680      690      700      710      720
GAG GTC TAC ACC TGC CAT GTG GAG CAT CCC AGC CTG AAG AGC CCC ATC
CTC CAG ATG TGG ACG GTA CAC CTC GTA GCG TCG GAC TTC TCG GCG TAG
E V Y T C H V E H P S L R S P I>
----- I-Ad β-2 domain -----

730      740      750      760      770
ACT GTG GAG TGG ACT AGT GGT GGC GGT AGC GGC GGT GGT GGT TCC
TGA CAC CTC ACC TGA TCA CCA CCG CCA CCG TCG CCG CCA CCA CCA AGG
T V E W T S G G G G S G G G G S>
-----><----- 24 amino acid linker -----

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FIGURE 27 (SHEET 3 OF 4)

```

      780      790      800      810
      .      .      .      .
GGT GGC GGC GGT TCT GGC GGT GGC GGT TCC TCC AGT GAA GAC GAC ATT
CCA CCG CCG CCA AGA CCG CCA CCG CCA AGG AGG TCA CTT CTG CTG TAA
G  G  G  G  S  G  G  G  G  S  S  S  E  D  D  I>
----->-----

820      830      840      850      860
      .      .      .      .      .
GAG GCC GAC CAC GTA GGC TTC TAT GGT ACA ACT GTT TAT CAG TCT CCT
CTC CCG CTG GTG CAT CCG AAG ATA CCA TGT TGA CAA ATA GTC AGA GGA
E  A  D  H  V  G  F  Y  G  T  T  V  Y  Q  S  P>
----- I-Ad α-1 domain -----

870      880      890      900      910
      .      .      .      .      .
GGA GAC ATT GCC CAG TAC ACA CAT GAA TTT GAT GGT GAT GAG TTG TTC
CCT CTG TAA CCG CTC ATG TGT GTA CTT AAA CTA CCA CTA CTC AAC AAG
G  D  I  G  Q  Y  T  H  E  F  D  G  D  E  L  F>
-----

920      930      940      950      960
      .      .      .      .      .
TAT GTG GAC TTG GAT AAG AAG AAA ACT GTC TGG AGG CTT CCT GAG TTT
ATA CAC CTG AAC CTA TTC TTC TTT TGA CAG ACC TCC GAA GGA CTC AAA
Y  V  D  L  D  K  K  K  T  V  W  R  L  F  E  F>
-----

970      980      990      1000      1010
      .      .      .      .      .
GGC CAA TTG ATA CTC TTT GAG CCC CAA GGT GGA CTG CAA AAC ATA GCT
CCG GTT AAC TAT GAG AAA CTC GGG GTT CCA CCT GAC GTT TTG TAT CGA
G  Q  L  I  L  F  E  P  Q  G  G  L  Q  N  I  A>
-----

1020      1030      1040      1050
      .      .      .      .
GCA GAA AAA CAC AAC TTG GGA ATC TTG ACT AAG AGG TCA AAT TTC ACC
CGT CTT TTT GTG TTG AAC CCT TAG AAC TGA TTC TCC AGT TTA AAG TGG
A  E  K  H  N  L  G  I  L  T  K  R  S  N  F  T>
----- I-Ad α-1 domain -----

1060      1070      1080      1090      1100
      .      .      .      .      .
CCA GCT ACC AAT GAG GCT CCT CAA GCG ACT GTG TTC CCC AAG TCC CCT
GGT CGA TGG TTA CTC CGA GGA GTT CCG TGA CAC AAG GGG TTC AGG GGA
P  A  T  N  E  A  P  Q  A  T  V  F  P  K  S  P>
----->----- I-Ad α-2 domain -----

1110      1120      1130      1140      1150
      .      .      .      .      .
GTG CTG CTG GGT CAG CCC AAC ACC CTT ATC TGC TTT GTG GAC AAC ATC
CAC GAC GAC CCA GTC GGG TTG TGG GAA TAG ACG AAA CAC CTG TTG TAG
V  L  L  G  Q  P  N  T  L  I  C  F  V  D  N  I>
-----

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FIGURE 28 (SHEET 2 OF 4)

```

390      400      410      420      430
GAG GTG GAC ACG GCG TGC AGA CAC AAC TAC GAG GGG CCG GAG ACC AGC
CTC CAC CTG TGC CGC ACG TCT GTG TTG ATG CTC CCC GGC CTC TGG TCG
E V D T A C R H N Y E G P E T S>
-----

440      450      460      470      480
ACC TCC CTG CCG CGG CTT GAA CAG CCC AAT GTC GCC ATC TCC CTG TCC
TGG AGG GAC GCC GGC GAA CTT GTC GGG TTA CAG CCG TAG AGG GAC AGG
T S L R R L E Q P N V A I S L S>
--- I-Ad β-1 domain -->----- I-Ad β-2 domain -----

490      500      510      520      530
AGG ACA GAG GCC CTC AAC CAC CAC AAC ACT CTG GTC TGT TCG GTG ACA
TCC TGT CTC CGG GAG TTG GTG GTG TTG TGA GAC CAG ACA AGC CAC TGT
R T E A L N H H N T L V C S V T>
-----

540      550      560      570
GAT TTC TAC CCA GCC AAG ATC AAA GTG CGC TGG TTC AGG AAT GGC CAG
CTA AAG ATG GGT CGG TTC TAG TTT CAC GCG ACC AAG TCC TTA CCG GTC
D F Y P A K I K V R W F R N G Q>
-----

580      590      600      610      620
GAG GAG ACA GTG GGG GTC TCA TCC ACA CAG CTT ATT AGG AAT GGG GAC
CTC CTC TGT CAC CCC CAG ACT AGG TGT GTC GAA TAA TCC TTA CCC CTG
E E T V G V S S T Q L I R N G D>
-----

630      640      650      660      670
TGG ACC TTC CAG GTC CTG GTC ATG CTG GAG ATG ACC CCT CAT CAG GGA
ACC TGG AAG GTC CAG GAC CAG TAC GAC CTC TAC TGG GGA GTA GTC CCT
W T F Q V L V M L E M T P H Q G>
-----

680      690      700      710      720
GAG GTC TAC ACC TGC CAT GTG GAG CAT CCC ACC CTG AAG AGC CCC ATC
CTC CAG ATG TCG ACC GTA CAC CTC GTA GGG TCG GAC TTC TCG GGC TAG
E V Y T C H V E H P S L K S P I>
----- I-Ad β-2 domain -----

730      740      750      760      770
ACT GTG CAG TCG ACT ACT GGT GCG GGT GCG AGC GCG GGT GGT GGT TCC
TGA CAC CTC ACC TGA TCA CCA CCG CCA CCG TCG CCG CCA CCA CCA AGG
T V E W T S G G G S G G G S>
----->----- 24 amino acid linker -----

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FIGURE 28 (SHEET 3 OF 4)

780 790 800 810
 GGT GGC GGC GGT TCT GGC GGT GGC TCC TCG AGT GAA GAC GAC ATT
 CCA CCG CCG CCA AGA CCG CCA CCG CCA AGG AGC TCA CTT CTG CTG TAA
 G G G G S G G G S S S E D D I>
 -----><-----

820 830 840 850 860
 GAG GCC GAC CAC GTA GGC TTC TAT GGT ACA ACT GTT TAT CAG TCT CCT
 CTC CCG CTG GTC CAT CCG AAG ATA CCA TGT TGA CAA ATA GTC AGA GGA
 E A D H V G F Y G T T V Y Q S P>
 ----- I-Ad α -1 domain -----

870 880 890 900 910
 GGA GAC ATT GGC CAG TAC ACA CAT GAA TTT GAT GGT GAT GAG TTG TTC
 CCT CTG TAA CCG GTC ATG TGT GTA CTT AAA CTA CCA CTA CTC AAC AAG
 G D I G Q Y T H E F D G D E L F>

920 930 940 950 960
 TAT GTG GAC TTG GAT AAG AAG AAA ACT GTC TCG AGG CTT CCT GAG TTT
 ATA CAC CTG AAC CTA TTC TTC TTT TGA CAG ACC TCC GAA GGA CTC AAA
 Y V D L D K K K T V W R L P E F>

970 980 990 1000 1010
 GGC CAA TTG ATA CTC TTT GAG CCC CAA GGT GGA CTG CAA AAC ATA GGT
 CCG GTT AAC TAT GAG AAA CTC GGG GTT CCA CCT GAC GTT TTG TAT CGA
 G Q L I L F E P Q G G L Q N I A>

1020 1030 1040 1050
 GCA GAA AAA CAC AAC TTG GGA ATC TTG ACT AAG AGG TCA AAT TTC ACC
 CGT CTT TTT GTG TTG AAC CCT TAG AAC TGA TTC TCC AGT TTA AAG TGG
 A E K H N L G I L T K R S N F T>
 ----- I-Ad α -1 domain -----

1060 1070 1080 1090 1100
 CCA GCT ACC AAT GAG GCT CCT CAA GCG ACT GTG TTC CCC AAG TCC CCT
 GGT CGA TCG TTA CTC CGA GGA GTT CCG TGA CAC AAG GCG TTC AGG GGA
 P A T N E A P Q A T V F P K S P>
 -----><----- I-Ad α -2 domain -----

1110 1120 1130 1140 1150
 GTG CTG CTG GGT CAG CCC AAC ACC CTT ATC TGC TTT GTG GAC AAC ATC
 CAC GAC GAC CCA GTC GCG TTG TCG GAA TAG ACG AAA CAC CTG TTG TAG
 V L L G Q P N T L I C F V D N I>

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FIGURE 28 (SHEET 4 OF 4)

1160 1170 1180 1190 1200
 TTC CCA CCT GTG ATC AAC ATC ACA TGG CTC AGA AAT AAT AAG TCA GT
 AAG GGT GGA CAC TAG TTC TAG TGT ACC GAG TCT TTA TCG TTC AGT CAG
 F P P V I N I T W L R N S K S V>

 1210 1220 1230 1240 1250
 ACA GAC GGC GTT TAT GAG ACC AGC TTC CTC GTC AAC CGT GAC CAT TCC
 TGT CTG CCG CAA ATA CTC TGG TCG AAG GAG CAG TTG GCA CTG GTA AGG
 T D G V Y E T S F L V N R D H S>

 1260 1270 1280 1290
 TTC CAC AAG CTG TCT TAT CTC ACC TTC ATC CCT TCT GAT GAT GAC ATT
 AAG GTG TTC GAC AGA ATA GAG TGG AAG TAG GGA AGA CTA CTA CTG TAA
 F H K L S Y L T F I P S D D D I>

 1300 1310 1320 1330 1340
 TAT GAC TGC AAG GTG GAG CAC TGG GGC CTG GAG GAG CCG GTT CTG AAA
 ATA CTG ACG TTC CAC CTC GTG ACC CCG GAC CTC CTC GGC CAA GAC TTT
 Y D C K V E H W G L E E P V L K>

 I-Ad α -2 domain -----
 1350 1360 1370 1380 1390
 CAC TGG GAA CCT GAG ATT CCA GGC CCC ATG TCA GAG CTG ACA GAA ACT
 GTG ACC CTT GGA CTC TAA GGT CCG GCG TAC AGT CTC GAC TGT CTT TGA
 H N E P E I P A P M S E L T E T>

 I-Ad α -TM domain -----
 1400 1410 1420 1430 1440
 GTG GTG TGT GGC CTG GCG TTG TCT GTG GGC CTT GTG GGC ATC GTG GTG
 CAC CAC ACA CCG GAC CCC AAC AGA CAC CCG GAA CAC CCG TAG CAC CAC
 V V C A L G L S V G L V G I V V>

 1450 1460 1470 1480 1490
 GGC ACC ATC TTC ATC ATT CAA GGC CTG CGA TCA GGT GGC ACC TCC AGA
 CCG TGG TAG AAG TAG TAA GTT CCG GAC GGT AGT CCA CCG TCG AGG TCT
 G T I F I I Q G L R S G G T S R>

 1500
 CAC CCA GGC CCT TTA TGA
 GTG GGT CCC GGA AAT ACT
 H P G P L *>
 - I-Ad α -TM domain ->

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FIGURE 29 (SHEET 1 OF 4)

```

      10      20      30      40      50
      .      .      .      .      .
CCACC ATG GCT CTG CAG ATC CCC AGC CTC CTC CTC TCA GCT GCT GTG GTG
GGTGG TAC CGA GAC GTC TAG GGG TCG CAG GAG GAG AGT CGA CGA CAC CAC
      M   A   L   Q   I   P   S   L   L   L   S   A   A   V   V>
<----- I-Ad β chain leader ----->

      60      70      80      90
      .      .      .      .
GTG CTG ATG GTG CTG AGC AGC CCA AGG ACC TTA AGT ATC TCT CAG GCT
CAC GAC TAC CAC GAC TCG TCG GGT TCC TGG AAT TCA TAG AGA GTC CGA
      V   L   M   V   L   S   S   P   R   T   L   S   I   S   Q   A>
----->-----<-----

    100      110      120      130      140
    .      .      .      .      .
GTT CAC GCT GCT CAC GCT GAA ATC AAC GAA GCT GGT CGT GCT AGC GGA
CAA GTG CGA CGA GTG CGA CTT TAG TTG CTT CGA CCA GCA CGA TCG CCT
      V   H   A   A   H   A   E   I   N   E   A   G   R   A   S   G>
----- OVA 323-339 ----->-----<-----

    150      160      170      180      190
    .      .      .      .      .
GGG GGC GGA AGC GGC GGA GGG GGA AAC TCC GAA AGG CAT TTC GTG GTC
CCC CCG CCT TCG CCG CCT CCC CCT TTG AGC CTT TCC GTA AAG CAC CAG
      G   G   G   S   G   G   G   G   N   S   E   R   H   F   V   V>
-- 10 amino acid linker -->----- I-Ad β-1 domain -----<-----

    200      210      220      230      240
    .      .      .      .      .
CAG TTC AAG GGC GAG TGC TAC TAC ACC AAC GGG ACC CAG CGC ATA CGG
GTC AAG TTC CCG CTC ACG ATG ATG TGG TTG CCC TGC GTC GCG TAT GCC
      Q   F   K   G   E   C   Y   Y   T   N   G   T   Q   R   I   R>
----->-----<-----

    250      260      270      280      290
    .      .      .      .      .
CTC GTG ACC AGA TAC ATC TAC AAC CCG GAG GAG TAC GTG CGC TAC GAC
GAG CAC TGG TCT ATG TAG ATG TTG GCC CTC CTC ATG CAC GCG ATG CTG
      L   V   T   R   Y   I   Y   N   R   E   E   Y   V   R   Y   D>
----->-----<-----

    300      310      320      330
    .      .      .      .
AGC GAC GTG GGC GAG TAC CCG CCG GTG ACC GAG CTC GGG CCG CCA GAC
TCG CTC CAC CCG CTC ATG GCG CCG CAC TGG CTC GAC CCC GCC GGT CTC
      S   D   V   G   E   Y   R   A   V   T   E   L   G   R   P   D>
----->-----<-----

    340      350      360      370      380
    .      .      .      .      .
GCC GAG TAC TGG AAC AGC CAG CCG GAG ATC CTG GAG CGA ACG CCG GCC
CGG CTC ATG ACC TTG TCG GTC GCG CTC TAG GAC CTC GCT TGC GCC CCG
      A   E   Y   W   N   S   Q   P   E   I   L   E   R   T   R   A>
----->-----<-----

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FIGURE 29 (SHEET 1 OF 4)

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FIGURE 29 (SHEET 2 OF 4)

```

390      400      410      420      430
GAG GTG GAC ACG GCG TGC AGA CAC AAC TAC GAG GGG CCG GAG ACC AGC
CTC CAC CTG TGC CGC ACG TCT GTG TTG ATG CTC CCC GGC CTC TGG TCG
E V D T A C R H N Y E G P E T S>
-----

440      450      460      470      480
ACC TCC CTG CCG CGG CTT GAA CAG CCC AAT GTC GCC ATC TCC CTG TCC
TGG AGG GAC GCC GGC GAA CTT GTG GGG TTA CAG CGG TAG AGG GAC AGG
T S L R R L E Q P N V A I S L S>
--- I-Ad β-1 domain -->----- I-Ad β-2 domain -----

490      500      510      520      530
AGG ACA GAG GCC CTC AAC CAC CAC AAC ACT CTG GTC TGT TCG GTG ACA
TCC TGT CTC CGG GAG TTG GTG GTG TTA GAC CAG ACA AGC CAC TGT
R T E A L N H H N T L V C S V T>
-----

540      550      560      570
GAT TTC TAC CCA GCC AAG ATC AAA GTG CGC TGG TTC AGG AAT GGC CAG
CTA AAG ATG GGT CGG TTC TAG TTT CAC GCG ACC AAG TCC TTA CCG GTC
D F Y P A K I K V R W F R N G Q>
-----

580      590      600      610      620
GAG GAG ACA GTG GGG GTC TCA TCC ACA CAG CTT ATT AGG AAT GGG GAC
CTC CTC TGT CAC CCC CAG AGT AGG TGT GTC GAA TAA TCC TTA CCC CTC
E E T V G V S S T Q L I R N G D>
-----

630      640      650      660      670
TGG ACC TTC CAG GTC CTC GTC ATG CTG GAG ATG ACC CCT CAT CAG GGA
ACC TGG AAG GTC CAG GAC CAG TAC GAC CTC TAC TGG GGA GTA GTC CCT
W T F Q V L V M L E M T P H Q G>
-----

680      690      700      710      720
GAG GTC TAC ACC TGC CAT GTG GAG CAT CCC AGC CTG AAG AGC CCC ATC
CTC CAG ATG TGG ACG GTA CAC CTC GTA GGG TCG GAC TTC TCG GCG TAG
E V Y T C H V E H P S L K S P I>
----- I-Ad β-2 domain -----

730      740      750      760      770
ACT GTG GAG TGG ACT AGT GGT GGC GGT GGC AGC GGC GGT GGT GGT TCC
TGA CAC CTC ACC TGA TCA CCA CCG CCA CCG TCG CCG CCA CCA CCA AGG
T V E W T S G G G G S G G G G S>
-----><----- 24 amino acid linker -----

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FIGURE 29 (SHEET 3 OF 4)

```

      780      790      800      810
      .      .      .      .
GCT GGC GGC GGT TCT GGC GGT GGC GGT TCC TCG AGT GAA GAC GAC ATT
CCA CCG CCG CCA AGA CCG CCA CCG CCA AGC AGC TCA CTT CTG CTG TAA
G G G G S G G G G S S S E D D I>
-----X-----

820      830      840      850      860
      .      .      .      .      .
GAG GCC GAC CAC GTA GGC TTC TAT GGT ACA ACT GTT TAT CAG TCT CCT
CTC CGG CTG GTG CAT CCG AAG ATA CCA TGT TGA CAA ATA GTC ACA GGA
E A D H V G F Y G T T V Y Q S P>
----- I-Ad α-1 domain -----

870      880      890      900      910
      .      .      .      .      .
GGA GAC ATT GGC CAG TAC ACA CAT GAA TTT GAT GGT GAT GAG TTG TTC
CCT CTG TAA CCG GTC ATG TGT GTA CTT AAA CTA CCA CTA CTC AAC AAG
G D I G Q Y T H E F D G D E L F>
-----

920      930      940      950      960
      .      .      .      .      .
TAT GTG GAC TTG GAT AAG AAG AAA ACT GTC TCG AGG CTT CCT GAG TTT
ATA CAC CTG AAC CTA TTC TTC TTT TGA CAG ACC TCC GAA GGA CTC AAA
Y V D L D K K K T V W R L P E F>
-----

970      980      990      1000      1010
      .      .      .      .      .
GGC CAA TTG ATA CTC TTT GAG CCC CAA GGT GGA CTG CAA AAC ATA GCT
CCG GTT AAC TAT GAG AAA CTC GGG GTT CCA CCT GAC GTT TTG TAT CGA
G Q L I L F E P Q G G L Q N I A>
-----

1020      1030      1040      1050
      .      .      .      .
CCA GAA AAA CAC AAC TTG GGA ATC TTG ACT AAG AGG TCA AAT TTC ACC
CGT CTT TTT GTG TTG AAC CCT TAG AAC TGA TTC TCC AGT TTA AAG TGG
A E K H N L G I L T K R S N F T>
----- I-Ad α-1 domain -----

1060      1070      1080      1090      1100
      .      .      .      .      .
CCA GCT ACC AAT GAG GGT CCT CAA GCG ACT GTG TTC CCC AAG TCC CCT
GCT CGA TCG TTA CTC CGA GGA GTT CGC TGA CAC AAG GGG TTC AGG CGA
P A T N E A P Q A T V F P R S P>
-----X----- I-Ad α-2 domain -----

1110      1120      1130      1140      1150
      .      .      .      .      .
GTG CTG CTG GGT CAG CCC AAC ACC CTT ATC TCG TTT GTG GAC AAC ATC
CAC GAC GAC CCA GTC GCG TTG TGG GAA TAG ACG AAA CAC CTG TTG TAG
V L L G Q P N T L I C F V D N I>
-----

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FIGURE 29 (SHEET 4 OF 4)

```

      1160      1170      1180      1190      1200
      .         .         .         .         .
TTC CCA CCT GTG ATC AAC ATC ACA TGG CTC AGA AAT AGC AAG TCA GTC
AAG GGT GGA CAC TAG TTG TAG TGT ACC GAG TCT TTA TCG TTC AGT CAG
P  P  P  V  I  N  I  T  W  L  R  N  S  K  S  V>
-----

      1210      1220      1230      1240      1250
      .         .         .         .         .
ACA GAC GGC GTT TAT GAG ACC AGC TTC CTC GTC AAC CGT GAC CAT TCC
TGT CTG CCG CAA ATA CTC TGG TCG AAG GAG CAG TTG GCA CTG GTA ACG
T  D  G  V  Y  E  T  S  F  L  V  N  R  D  H  S>
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      1260      1270      1280      1290
      .         .         .         .
TTC CAC AAG CTG TCT TAT CTC ACC TTC ATC CCT TCT GAT GAT GAC ATT
AAG GTG TTC GAC AGA ATA GAG TGG AAG TAG GGA AGA CTA CTA CTG TAA
F  H  K  L  S  Y  L  T  F  I  P  S  D  D  D  I>
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1300      1310      1320      1330      1340
      .         .         .         .         .
TAT GAC TGC AAG GTG GAG CAC TGG GGC CTG GAG GAG CCG GTT CTG AAA
ATA CTG ACG TTC CAC CTC GTG ACC CCG GAC CTC CTC GGC CAA GAC TTT
Y  D  C  K  V  E  H  W  G  L  E  E  P  V  L  K>
----- I-Ad α-2 domain -----

      1350      1360      1370      1380
      .         .         .         .
CAC TGG GAG GAA GAA GAG TAC ATG CCG ATG GAA TGA
GTG ACC CTC CTT CTT CTC ATG TAC GGC TAC CTT ACT
H  W  E  E  E  E  Y  M  P  M  E  *>
-----> EE tag ----->

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